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Pathology

PRENATAL DIAGNOSIS OF BETA THALASSEMIA AND OTHER HAEMOGLOBINOPATHIES - INVASIVE APPROACH IN A TERTIARY CARE HOSPITAL

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ABSTRACT **Introduction:** Thalassemia and other hemoglobinopathies are common genetic disorders affecting children worldwide which can be prevented by antenatal screening and carrier detection. These disorders create huge financial and emotional deficit on the affected individuals and their families thereby making timely diagnosis crucial. **Aim:** To find the prevalence of carriers of thalassemia and other haemoglobinopathies in pregnant females within 20 weeks of pregnancy and their husbands so as to identify the couples at risk. Genetic counselling and prevention of birth of thalassemia child by offering prenatal diagnosis and selective abortion of the fetuses affected with thalassemia. **Materials and methods:** A prospective study was conducted among 100 antenatal patients with microcytic hypochromic anaemia before 20 weeks of gestation during the study period of two years. Partners of all pregnant females who turned out to be hemoglobinopathy carriers were also screened by HPLC. Prevention of birth of thalassemia child by offering genetic counseling and prenatal diagnosis was done. Results: In the present study, out of 100 antenatal patients, we found 18 females to be beta thalassemia carriers and one patient with sickle cell trait. The partners of these females were also screened. Three couples with both parents as beta thalassemia carriers opted for Chorionic Villus Sampling and the fetus tested negative for homozygous trait. **Conclusion:** Prenatal screening and creating awareness during antenatal check-ups will help in timely diagnosis and prevention of birth of severely affected thalassemia child. All pregnant women with microcytic hypochromic anemia must be screened preferably before 20 weeks of gestation during their first antenatal visit to the hospital.

KEYWORDS : Prenatal diagnosis, Thalassemia, ANC, Sickle cell disease, Chorionic villus sampling, Foetus

INTRODUCTION

Beta – thalassemia major is an autosomal recessive disorder caused by decreased or absent beta globin chain synthesis. There are an estimated 7500 - 12,000 babies with β-thalassemia major born each year in the country with life-threatening anemia and requiring regular blood transfusion for survival. (1)

High incidence of thalassemia is reported across the land of Mediterranean, Arabian Peninsula, Central Asia and India. In India, communities where beta-thalassemia is prevalent include the Punjabis, Sindhis, Gujaratis, Mahars, Saraswats, Kolis and Bengalis. The percentage of cases ranges from 1 to 17 %. (1)

Sickle cell disease is caused by a single base-pair point mutation (GAG to GTG) which results in the replacement of the glutamic acid to Valine at the 6th position of the beta-chain of hemoglobin denoted as hemoglobin S (HbS). (2)

High-performance liquid chromatography (HPLC) is the most frequently used method for the identification of abnormal hemoglobin chains as well as quantitative assessment of hemoglobin variants.

Pregnant females visiting for ANC during the first trimester are tested using chorionic villus sampling (CVS). Reverse dot blot hybridization, amplification refractory mutation system (ARMS) and DNA sequencing are used for evaluating the hemoglobin defects. Cordocentesis and fetal blood analysis using HPLC are performed for prenatal detection in the second trimester.

MATERIALS AND METHODS

A prospective study was carried out on 100 cases of pregnant females with microcytic hypochromic anaemia, attending the antenatal clinic of the hospital before 20 weeks of gestation during the study period of two years.

Inclusion Criteria:

1. Antenatal patients with microcytic hypochromic anemia visiting the tertiary care center within 20 weeks of pregnancy were included in the study.

2. Known cases of pregnant females with thalassemia coming for antenatal checkup within 20 weeks of gestation were included.

Exclusion criteria: Patients who had received blood transfusion in the past 1 month were excluded from the study.

The clinical details of the pregnant females within 20 weeks of gestation were recorded on a predesigned pro forma. Under aseptic precautions, 2 mL blood sample was collected in EDTA vacutainer after informed consent. The blood samples were subjected for complete blood count on XN 1000, 6-part differential cell counter along with peripheral blood smear examination for microcytic hypochromic anemia.

Mentzer's index was calculated by dividing the MCV by the RBC count. If MCV divided by RBC was less than 13, thalassemia was said to be more likely and if greater than 13, then iron-deficiency anemia was the more probable cause. Screening for hemoglobinopathies by HPLC using D10- Biorad was performed on the EDTA sample. Subjects with HbA2 levels of 3.5 percent and above were considered to have thalassemia trait.

Partners of all pregnant females who turned out to be hemoglobinopathy carriers were screened by HPLC. If both partners were found to be carriers, the next step was to prevent the birth of thalassemia child by offering genetic counseling, prenatal diagnosis and selective abortion of the fetuses affected with thalassemia. Based on weeks of gestation, the invasive method of diagnosis was suggested.

Chorionic villus sampling (CVS) was performed between 10 to 12 weeks of gestation. Diagnosis was obtained by 24 hours and if termination is considered, it could be done in the first trimester safely. After 15th week of gestation, Amniocentesis would be suggested. At 18 - 20 weeks of gestation, cordocentesis using cord blood would be used for prenatal diagnosis.

OBSERVATION AND RESULTS

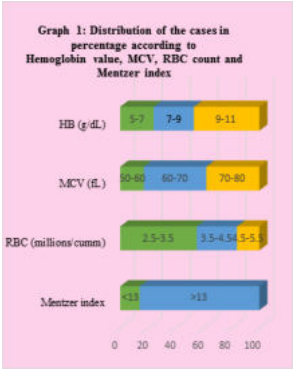
The present study comprising of 100 ANC cases showed that the most

common age group among the antenatal patients in the study was 26-30 years (53%). Maximum number of cases (54%) presented at 15 to 20 weeks of gestation. The least number of patients, i.e., 6 pregnant females were found in the age group of 20-25 years. Only 5% of the antenatal cases presented to the OPD at 5 to 10 weeks of gestational period.

TABLE 1 PROFILE DETAILS OF STUDY POPULATION.

Categorical Variables		Total number (n =100)	Percentage (%)
Age	20-25 years	41	41
	26- 30 years	53	53
	31-35 years	6	6
Weeks of gestation	5-10	5	5
	10-15	41	41
	15-20	54	54

Majority of ANC patients were found to have low hemoglobin value in the range 9 - 11 g/dL (47 cases). Majority of the cases presented with MCV in the range 60-70 fL (45%) and RBC count of 2.5-3.5 millions/cumm (55%)



24 % of the cases presented with hemoglobin value in the range of 5 to 7 g/dL, 29 % in the range 7 to 9 g/dL and 47 % had hemoglobin measuring between 9 to 11 g/dL. Only 17 % cases had very low MCV value of 50-60 fL and for the majority of cases, i.e. 45 % the MCV varied between 60 to 70 fL. In 55 % of the cases, we found RBC count in the range of 2.5 to 3.5 million/ cumm and for 16 % cases between 4.5 to 5.5 million/ cumm. In 14 % patients, Mentzer index calculated was below 13 and in 86% it was above 13.

Out of the 100 ANC cases, 18 females were diagnosed as carriers of beta thalassemia trait on HPLC and 1 female was identified as carrier of Sickle cell disease. The patient with sickle cell disease had HbS value of 8.8 %.

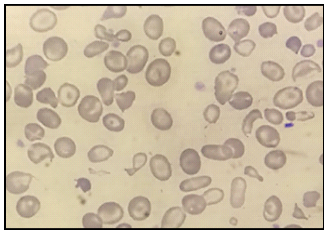


Figure 1: Peripheral smear from a beta-thalassemia major patient showing marked hypochromasia, microcytosis and anisopoikilocytosis. Target cells, tear drop cells and occasional polychromatophilic cells are also seen. (Field's stain, x1000)

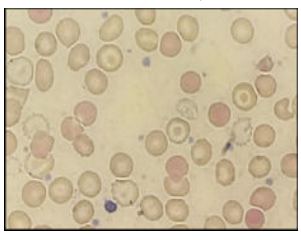


Figure 2: Peripheral smear from a patient with beta thalassemia minor showing microcytosis, poikilocytosis and target cells. (Field's stain, x1000)

All the patients identified as beta thalassemia carriers had HbA2 value increased more than 3.5% ranging from 3.5% to 6.5%. On HPLC, majority of the ANC cases, that is 59%, were found to have HbA2 in the range 2.0 to 3.4%.

History of consanguinity was elicited in only one couple among the 100 cases. Only two pregnant women out of 19 gave history of hemoglobin disorders in their family. This could be possible as a result of unawareness or can be attributed to the social stigma that such genetic disorders carry in our society.

Husbands of the 19 pregnant females were screened for hemoglobinopathy and HPLC was suggested. Three husbands turned out to be Beta Thalassemia Carriers. None of the three couples had received any pre-marital screening.

Genetic counseling was done for these three couples and prenatal diagnosis was recommended. Risks associated with birth of Beta thalassemia child were explained to the parents and the couples were referred for Chorionic villous sampling.

Chorionic villous sampling of these patients was performed at 10-12 weeks of gestation after counseling and obtaining written consent. The Chorionic villous tissue DNA analysis in all three patients showed absence of homozygosity for Beta thalassemia major in the fetus. Hence, the pregnancy was continued till term.

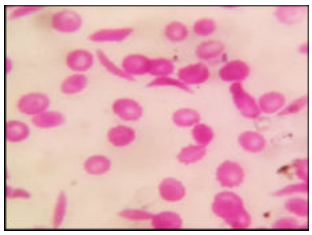


Figure 3: Peripheral smear from a patient with sickle cell disease showing presence of sickle cells. (Field's stain, x1000)

DISCUSSION

Thalassemia and sickle cell diseases are common genetic diseases across the world as well as in the Indian subcontinent growing the burden on health care services and impairing quality of life. As stated by World Health Organization, the extent of world population affected by thalassemia and allied erythrocyte disorders is 4.5% across the globe. [3] Thalassemia is an inherited disorder with two main classifications: Alpha-thalassemia and Beta-thalassemia. Decreased synthesis or absence of the beta globin chain in the hemoglobin molecule results in Beta-thalassemia. There are three types of Beta thalassemia: Thalassemia Minor, Thalassemia Intermediate and Thalassemia Major or Cooley's anemia.

The findings of the present study revealed a mean haemoglobin value of 8.8 ± 1.85 g/dL in pregnant females with beta thalassemia trait and 8.1±1.1g/dL in those without beta thalassemia trait. Similarly, mean value of MCV was 62.1 ± 7.2 fL in thalassemia carriers and 69.3 ± 6.8 fL in those who were not carriers. Therefore, the haemoglobin and MCV levels are lower in beta thalassemia trait pregnant females than other antenatal care patients with anaemia due to causes other than haemoglobin disorders, comparable to the studies conducted by Sharma et al and Mendiratta et al. [4, 5] However, study conducted by Tasneem et al showed lower haemoglobin values in non- beta thalassemia carriers and is in discordance with our study. [6] (Table 2) Conversely, the RBC count was 4.9 ± 0.5 million/cumm which was higher in pregnant females with beta thalassemia trait compared to the non- beta thalassemia trait group (3.34 ± 0.6 million/cumm) which is in accordance with studies done by Sharma et al, Tasneem et al and Mendiratta et al. [4,5,6]

In our study, on comparing the Mentzer index, a mean ratio of 12.7 ± 2.2 was obtained in beta-thalassemia carriers, lesser than 21.54 ± 4.79 in non-beta thalassemia carriers. Hence, we concluded that Mentzer index can be considered as quite a reliable tool and patients can be advised HPLC. These findings are similar to the interpretation drawn from studies conducted by Zafar et al and Bhushan et al with mean ratio of 12.2 ± 5.3 and 11.91± 5.05 in thalassemia group. [7,8] Similar to the present study, the mean ratio in the control group was 21.0 ± 10.5 and 21.0 ± 10.5 in these studies.

The gold standard for detecting thalassemia and haemoglobinopathies is estimation of HbA2 levels and other variants on HPLC analysis.

Table 3: Correlation of HbA2 values with other studies

Different studies	HbA2 value	
	BTT group	Non-BTT group
Sharma et al	4.3 ± 1.8	2.6 ± 0.74
Mondal et al	4.1 ± 1.7	2·7 ± 0.4
Urrechaga et al	4.7 ± 0.19	1.6 ± 0.26
Our study	4.8 ± 0.73	2.4 ± 0.54

We observed that the mean HbA2 levels in ANC patients with beta thalassemia trait was 4.8 ± 0.73 % whereas it was 2.4 ± 0.54 in those without beta thalassemia trait which is in accordance with the results of studies done by Sharma et al, Mondal et al and Urrechaga et al. (4, 9, 10)

Bukhanwala et al found 3.38% (102) beta thalassemia carriers and sickle cell trait was found in 1.5 % (46) of the 3,009 antenatal patients in the study. (11)

11 out of 14 couples at risk opted for prenatal diagnosis and medical termination of pregnancy was carried out for three foetuses found to have homozygous β-thalassemia.

In a study by Colah R et al, 61,935 antenatal patients were screened over a span of 7 years, 713 females were diagnosed as beta thalassemia carriers and HPLC was done for their husbands. 37 couples were found to be at risk of giving birth to a thalassemia major child.15 couples opted for prenatal interventional testing and 4 chose to terminate the pregnancy. 22 out of the 37 couples did not follow up after the process of genetic counselling. [12]

TABLE 2: Comparison of red blood cell indices (Mean ± SD) in Antenatal care females with other studies

Different studies	Hemoglobin (g/dL)		MCV (fL)		RBC(million/cumm)	
	BTT	Non-BTT	BTT	Non-BTT	BTT	Non-BTT
Sharma et al	8.7 ± 2.10	10.2 ± 1.40	63.2 ± 6.5	79.5 ± 4.9	5.52 ± 0.62	5.23 ± 0.7
Tasneem et al	9.8 ± 0.96	9.09 ± 1.02	61.6 ± 7.18	71.6 ± 6.29	4.98 ± 0.74	3.97 ± 0.4
Mendiratta et al	11 ± 1.80	11.5 ± 1.68	75.9 ± 11.3	88.6 ± 8.21	4.60 ± 0.72	4.04 ± 0.5
Our study	8.1±1.10	8.8 ± 1.85	62.1 ± 7.20	69.3 ± 6.80	4.9 ± 0.50	3.34± 0.6

In this present study, 18 out of the 100 pregnant females we found to have beta thalassemia trait and one female was sickle cell trait. On screening the husbands, three couples turned out to have both partners as beta thalassemia carriers. All three cases were counselled and they opted for chorionic villus sampling. The foetuses were tested in view of maternal as well as paternal genotype for homozygous trait and were reported as negative with pregnancy followed until term.

These findings are in accordance with other studies carried out on antenatal patients such as, Chareonkul P et al and Colah R et al. [12,13] Chorionic villus sampling was preferred in this study as it can be performed early during the first 10-12 weeks of gestation, lower risk of leakage of amniotic fluid and has lower infection rate when compared to amniocentesis. The incidence of foetal mortality is also much lower in chorionic villus sampling than with other methods and the result is available within one week.

Chareonkul P et al conducted a study in which 3,739 pregnant females participated in the program and 931 women were carriers of beta-thalassemia trait. The husbands were screened as well and it revealed that 20 couples could probably have thalassemia affected child. 12 couples went for cordocentesis, out of which three were documented to have homozygous trait and selective abortion was performed. [13]

With the help of a proper plan for systematic screening of antenatal population and identifying carriers of thalassemia and other hemoglobinopathies, prenatal diagnosis helsp in thalassemia prevention. For example, the Republic of Cyprus has reported significant decrease in the incidence of beta thalassemia major by 96% by the method of genetic counseling and pre-natal check-up. [14]

CONCLUSION

Thalassemia and other hemoglobinopathies are genetic disorders affecting red blood cells and posing a national health burden. Lack of awareness among patients is responsible for birth of children with beta thalassemia major in spite of these disorders being potentially preventable.

Identification of carriers is obligatory in populations in which both α- and β-thalassemia are known to be prevalent. The beginning step for the identification of hemoglobinopathy carriers is evaluating hematology parameters and discussing the clinical history with the diagnostic physician. HPLC should be used as a gold standard test for diagnosis of thalassemia and other hemoglobinopathies with satisfactory sensitivity and specificity. HbA2 levels would also be beneficial in identifying beta-thalassemia major patients from the carriers. These findings together contribute in genetic counselling of the ANC patients and encourage couples to opt for prenatal diagnosis. The couple at risk of having a severely affected thalassemia child can be explained the clinical manifestations, life expectancy and management of thalassemia major which includes lifelong red blood cell transfusions at regular intervals and iron chelation therapy. The couple can be explained the option of selective abortion and will be able to take an informed decision about the birth of the foetus and hence we can prevent the birth of severely affected foetuses with beta thalassemia and other haemoglobinopathies.

DISCLOSURE OF CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

ETHICAL APPROVAL

The study was approved by the Institutional Ethical Committee.

REFERENCES:

[1] Colah, R B et al. "Invasive & non-invasive approaches for prenatal diagnosis of haemoglobinopathies: experiences from India." The Indian journal of medical research vol. 134,4 (2011): 552-60.

[2] Hoban, M.D.; Orkin, S.H.; Bauer, D.E. Genetic treatment of a molecular disorder: Gene therapy approaches to sickle cell disease. Blood 2016, 127, 839–848.

[3] Mendiratta SL, Mittal M, Naaz F, Singh S, Anand S. Role of thalassemia screening in prevention and control of thalassemia - a 5-year experience. Int J Reprod Contracept Obstet Gynecol 2016; 5:3107-11

[4] Sharma, Anju & Uppal, Neha & Kukreja, Sahiba & Kaur, Mandeep & Kaur, Sukhjeet. (2020). Screening of Thalassemia in pregnant females visitng tertiary hospital in Amritsar. International Journal of Clinical Biochemistry and Research. 7. 226-231. 10.18231/j.ijcbr.2020.049.

[5] Mendiratta, S.L., Bajaj, S., Popli, S. et al. Screening of Women in the Antenatal Period for Thalassemia Carrier Status: Comparison of NESTROFT, Red Cell Indices, and HPLC Analysis. J. Fetal Med. 2, 21–25 (2015).

[6] Tasneem, Sabahat & Sultana, N. & Snover, A. & Alam, K. (2016). Clinical utility of red blood cell count, red cell distribution width: Will it provide more accurate differentiation of Beta Thalassemia trait and iron deficiency anemia in pregnancy. 41. 424-427.

[7] Bhushan R, Shukla S, Singh D, Trivedi SS et al. Reliability of Different RBC Indices to Differentiate Between Beta Thalassemia Trait and Iron Deficiency Anemia During Antenatal Screening. World J Pathology. Vol. 10. 2018/ ISSN-2277-3460

[8] Zafar, Majida & Tabassum, Arfa & Cheema, Quratulain & Mazhar, Syeda. (2019). Role of Red Cell Distribution Width and Mentzer Index in Differentiating Iron Deficiency Anemia from Anemia Due to β Thalassemia Trait. Journal of South Asian Federation of Obstetrics and Gynaecology. 11. 297-300. 10.5005/jp-journals-10006-1718.

[9] Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: A 10-year high-performance liquid chromatography study of 119,336 cases. Asian J Transfus Sci. 2016;10(1):105-110. doi:10.4103/0973-6247.175424

[10] Urrechaga E, Evaluation of the Primus Ultra2 HPLC system for HbA2 measurement and β-thalassemia screening. Biochemia Medica 2008;18(3):351–60

[11] Bhukhanwala DS, Sorathiya SM, Sawant P, Colah R, Ghosh K, Gupte SC. Antenatal screening for identification of couples for prenatal diagnosis of severe hemoglobinopathies in Surat, South Gujarat. J Obstet Gynae India. 2013: 63(2); 123-7.

[12] Colah R, Surve R, Wadia M, Solanki P, Mayekar P, Thomas M, GorakshakarA, Dastur A, Mohanty D. Carrier screening for beta-thalassemia during pregnancy in India: a 7-year evaluation. Genet Test. 2008 Jun;12(2):181-5. doi:10.1089/gte.2007.0066. PubMed PMID: 18439108.

[13] Chareonkul P, Kraisin J. Prevention and control of thalassemia at Saraburi Regional Hospital. J Med Assoc Thai. 2004 Jan;87(1):8-15. PMID: 14971529.

[14] Buki MK, Qayum I, Siddiqui N. Prevalance and preventive measures for thalassemia in Hazara region of NWFP Pakistan. J Pak Med Assoc. 1998; 10;28-31.