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Antenatal screening for Thalassemia minor among conceiving females: A preventive measure

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ABSTRACT

Background: Beta Thalassemia is one of the most common global health concerns worldwide. It affects a large population in Pakistan, too, thereby increasing the financial burden. Several screening procedures have been proposed to lessen the cost burden associated with Beta Thalassemia. This study focuses on studying the occurrence of beta-thalassemia trait among pregnant ladies, the commonest mutations among Beta Thalassemia Trait cases, and defining the hematological parameters to overcome this expensive burden.

Materials and Methods: Blood was collected via venipuncture to carry-out CBC (Complete Blood Count) and H.B. (Hemoglobin) Electrophoresis is used to detect beta-thalassemia minor. In this study, the main CBC parameters to screen BTT included MCV (≤75fl), MCH (≤25pg), RBCs (≥4.50 million), and Hemoglobin (≤12g/Dl), whereas Hb electrophoresis confirmed the final diagnosis. The cut-off values for the final confirmation of BTT through Hb electrophoresis were >3.5% HBA2 and <95% HBA. Statistical tests used during the study included Mean and Standard deviation. Tetra-arm multiplex PCR was carried out to detect mutations.

Results: Thalassemia minor was detected in 15 out of 509 conceiving females present in our study cohort, thus overall incidence rate being 2.9%. Moreover, the most reliable parameters for screening beta-thalassemia minor included MCV, MCH, RBCs, and RDW. Iron Deficiency Anemia (IDA) didn't hinder the accurate diagnosis of the beta-thalassemia minor. Moreover, our data revealed the IVS 1-5(G-C) (4 samples) and FSc 8-9 (+G) (4 samples) to be the commonest mutations among carrier females. However, CD 30 mutation was found in 2 samples. However, Primers were designed for the most commonly reported mutations in Pakistan including F.Sc 8/9, IVS 1- 5, 619bp deletion, CD 16, and CD 30.

Conclusion: Extensive screening strategies and detailed genetic counselling are needed to identify the risk and genetic epidemiology of Beta Thalassemia in Pakistan.

1. Introduction

Beta Thalassemia is one of the autosomal recessive inherited disorders[1,2] characterized by a severely low Hemoglobin[3,4] or fewer number of erythrocytes (RBCs) in the bloodstream. The commonest symptoms include weakened bones, hypoxia, pallor,[4–6] and jaundice. Other associated anomalies observed among patients of β-thalassemia include splenomegaly, growth retardation, Bone Deformation,[6] and Hepatomegaly.[7] The disease is most prevalent in Mediterranean areas, South-East Asia, and the Middle East.[8–10] The main causative agent behind the

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inherited disorder is an absent or reduced synthesis of the beta-globin chain resulting from substitution mutation in the β-globin gene.[11–13] Patients with beta thalassemia have a life expectancy of up to 30 years if left untreated. Excess iron is built up due to continuous blood transfusion in patients of beta thalassemia. Iron chelation therapy (ICT) is employed to eliminate the excess iron from the body.[14,15] Apart from a blood transfusion, the most effective treatment strategy employed against B.T. is bone marrow transplant therapy requiring HLA (Human Leukocyte Antigen) compatibility between donor and recipient's blood. However, it is more costly, and that's why families and the health sector endure a severe economic burden that needs to be reduced by adopting unique prevention therapies.[16,17]

Implementation of cost-effective screening strategies to lessen the overall incidence of BTM (Beta-Thalassemia Major) in society must be a priority.[18] Globally, several anti-thalassemia screening programs played a pivotal role in decreasing the incidence of beta-thalassemia[19] (Figure 1). However, in Pakistan, some collective efforts at the national level are needed regarding the prevention and management of β-thalassemia. According to an estimate, around 60,000 babies are born worldwide with beta-thalassemia per year, whereas around 1.5% of individuals suffer from BTT worldwide[20] (Figure 1). In Pakistan, the prevalence of beta-thalassemia minor is between 5 and 8%.[18]

Concerning prevalent mutations, over 218 beta-thalassemia mutations have been reported worldwide, with IVS 1-5 being the most common B.T. mutation. So far, 20 mutations are found to be responsible for about 90% of the beta-globin gene disorder.[21] According to literature, IVS 1-5 (G-C) (with an overall incidence rate of 37.7%),[22] and F.Sc 8/9 (+G) (21.1%), 619bp (12.9%), IVS 1-1 (9.5%) are the most commonly occurring mutations among the citizens of Pakistan. Furthermore, other commonly reported mutations include IVS 1-1 (G-T), CD 16, CD 30, and CD41/42, with an overall incidence of 9.17%.[21]

Complete Blood Count (CBC), a routinely performed laboratory test, is a key parameter for the screening of Beta Thalassemia Trait. On the other hand, Hb electrophoresis is performed for the ultimate diagnosis of BTT.[23] The prenatal test is conducted during the 12^{th} week of pregnancy to give an informed choice to the patients regarding continuance of pregnancy.[24] The current study was executed to find out the prevalence of thalassemia minor among pregnant females.

The objectives of the study are as follows.

1. Investigation of the frequency of beta-thalassemia trait among local females.
2. To check the reliability of Mentzer's test for the screening of BTT.
3. Determination of the commonest mutation among thalassemia minor individuals.

2. Material and Methods

Current study has been conducted in the antenatal Outpatient Departments (OPDs) of Pakistan Institute of Medical Sciences (PIMS) Islamabad, Benazir Bhutto Hospital (BBH), and General Cantonment Hospital Rawalpindi. However, the laboratory work was performed at the Punjab Prevention thalassemia Program in Rawalpindi. Due to the involvement of human subjects, the approval for the study on the present topic was taken by the ethical committee of PMAS AAUR. A questionnaire was prepared to collect the bio-data of patients consisting of necessary information about patient including patient's name, identification number, ethnicity, age, and CBC. After obtaining written informed consent from each patient, all data was obtained prior to sample collection. Whole blood samples were drawn from pregnant women via venipuncture in order to check for thalassemia minor. Venous blood was collected for the current study. 4-5 ml of venous blood was collected using a fine sterilized syringe (BD, Germany). Sample blood was taken in special vacutainers containing EDTA (Ethylene Diamine Tetra Acetic Acid Potassium Salt) to prevent the blood sample from clotting. The most important parameters to screen for BTT included Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Red Blood Cells (RBC), Red Cell Distribution width (RDW), and Hemoglobin (Hemoglobin).

2.1. Hematological studies

2.2. Inclusion criteria

A sample of n=509 pregnant females was taken to find out the prevalence of beta-thalassemia trait among conceiving females during a study period from 18^{th} of Oct 2020 till 18^{th} of March 2021 (with the age ranging from 17-65). However, samples with MCV < 75fl, MCH < 25pg, RBCs>4.50 million, and Hemoglobin < 12g/dl were sent for further analysis through Hb electrophoresis. Samples were run on Mindray 3000 plus –B.C. Hematology analyzer for CBC analysis. We also used Mentzer's test (MCV/RBCs) to check its reliability for the screening of BTT.[25]

2.3. Hemoglobin electrophoresis and polymerase chain reaction

Samples were run on a capillary machine by the automation system of Sebia2 Flex Piercing for the final detection of BTT. The Standard Deviation and Mean of all the parameters were analyzed by using SPSS software. Apart from conducting hematological studies, samples were also subjected to Multiplex Tetra-Arm Polymerase Chain Reaction for mutational analysis. The extracted DNA was stored at -80 °C. DNA samples with minute quantities were discarded. 10 samples were tested to identify the type of

mutation present in selected individuals. Primers for the already reported mutations were designed, including IVS I-5 (G-C), FSC 8-9 (+G), -619 bp, Cd 16, Cd 30, and Cd 41/42. Extracted DNA was quantified by Nanodrop or 0.8% Agarose Gel.

3. Results

3.1. Incidence of beta-thalassemia minor

In the present study, n=15 individuals were detected with Beta thalassemia trait through Hb electrophoresis. Hence, the frequency of the beta-thalassemia trait in our current study was revealed to be 2.9% out of the total number (n=509). Mean and Standard Deviation values of all CBC parameters among pregnant ladies are given in Tables 1 and 2.

CBC parameters or Red Cell Indices played the most reliable role in detecting BTT. During current study, MCV, MCH, RDW, and RBCs were found to be the most dependable parameters as compared to MCHC and HCT in the detection of beta-thalassemia traits. The Hemoglobin levels in pregnant women with only the beta-thalassemia trait were from 10.7- 11.4g/dl. On the other hand, among women experiencing the coexistent condition (IDA and BTT), Hemoglobin was found in the range of 8.4g/dl and 9.8g/dl. MCV (Mean Corpuscular Volume) and MCH (Mean Corpuscular Hemoglobin) were also found to be significant in the detection of BTT. Other than that, there was no interference of IDA on the quantitative expression of HbA1 and HBA2.

3.2. Hemoglobin and Beta-thalassemia trait

IDA must be removed to eliminate the possibility of a false diagnosis of BTT because, according to some studies, the presence of IDA can hinder the accurate diagnosis of BTT.[26,27] However, in the current study, we didn't find any case where IDA interfered with the correct diagnosis of BTT. Moreover, we observed a variation in Hemoglobin levels among BTT individuals which may be due to presence of IDA.

Mentzer's index (one of the indices used for the detection of BTT)[25] has also been employed in the current study to analyze its reliability in the detection of beta thalassemia trait. According to the current study, 12 individuals with BTT were found to have Mentzer's index lower than 13, whereas 3 individuals with BTT were detected with MI more than 13. The sensitivity and specificity of the test have also been calculated during the study conducted on conceiving females. Specificity was 100%, indicating the test to be significantly reliable for the detection of BTT.

Molecular analysis has also been executed to identify the most prevalent mutations among tested individuals. N=10 samples were screened for the prevalent β-Thalassemia mutations in the Pakistani population, including IVS I-5 (G-C), FSC 8-9 (+G), -619 bp, Cd 16, Cd 30, and Cd 41/42. 4 out of 10 samples were reported with FSC 8/9 (+G), 4 with IVS 1/5 (G-C), and 2 out of 10 samples were detected with CD 30.

All carrier ladies' husbands were also tested for carrier status. None of them was discovered to be carriers. Surprisingly, physicians were also unaware of the diagnostic parameters for the detection of BTT. Most clinicians advised Hemoglobin electrophoresis without considering more important parameters apart from Hb for the detection of BTT. So, the provision of awareness must be incorporated in antenatal clinics among physicians as well to reduce the burden of elevated B.T. cases from Pakistan.

4. Discussion

The current study found disease prevalence, which validates earlier research on the incidence of thalassemia minor among pregnant women. For instance, as per a study conducted by Iqbal et al. in twin cities of Pakistan,[28] prevalence of Beta thalassemia trait among pregnant ladies was 4.05%. Similarly, another group of researchers,[29] studied conceiving females with age 18-39 with regard to antennal screening in the Pakistan Institute of Medical Sciences and found the overall BTT prevalence being 4.9. The results depicted by our study are similar to those of already conducted studies, thereby further strengthening the point.

According to the current study, the frequency of beta thalassemia trait among conceiving females (with the age group of 17-65) was found out to be 2.9%. Apart from it, it was not difficult to diagnose BTT in pregnant ladies suffering from Iron deficiency anaemia (IDA) indicating that BTT could be diagnosed despite the presence of IDA. Other than that, the variation in Hemoglobin level was found due to the presence of other complications of pregnancy, including Iron Deficiency Anemia.

Haematological parameters have played a significant part in the diagnosis of BTT. For instance, a study carried out by Bencaiova et al. (2006)[30] suggests that MCV is the most suitable parameter to differentiate beta-thalassemia trait from Iron Deficiency Anemia. A comparative analysis of CBC parameters for BTT screening is given in Table 3.

The new analysis validated the bulk of previous studies that showed the vital role of CBC parameters in the detection of BTT. Regarding prevalence, in another study executed by Rizwan et al.,[35] at the Antenatal Ward Department of Obstetrics and Gynaecology, Liaquat University Hospital Hyderabad, for a period of 2 years, the frequency was 8.5%.However, some studies have shown contradictory results as compared to our study. For instance, Qadir et al. (2017) studied conceiving ladies with Hgb < 10.5 g/dl in Khyber teaching Hospital and found the BTT frequency being 56.1%.[36] This disparity may be attributed to differences in research areas, which have been reported to

Table 1: A comparison among variety of Hematological condition observed in pregnant females through mean values

Hematological parameters	IDA (Iron deficiency Anemia)	BTT with co-existence of IDA	Only BTT	Controls
MCV (fl)	69.29268	60.4857	60.8222	79.0989
MCH (pg)	23.30774	19.9714	20.4444	27.9759
HCT (%)	27.18065	28.3333	31.0222	33.0220
MCHC (g/dl)	35.33446	33.3000	33.7333	37.3748
HGB (g/dl)	8.95	9.5500	10.6889	11.6266
RBCS (106/μl)	3.952381	4.7457	5.1811	4.1716
RDW-CV (%)	16.54333	16.9714	17.5778	14.7418
RDW-SD	40.575	37.4889	36.4556	42.1913

Table 2: Statistical analysis of CBC parameters in BTT individuals

		Mean Corpuscular Volume	Mean Corpuscular Hemoglobin	Hemo globin	Red Blood Cells	Mean Corpuscular Hemoglobin Concentration
N	Valid	16	16	16	16	16
	Missing	0	0	0	0	0
Mean		60.9563 (fl)	20.30 (Pg)	10.13 g/dl	5.00× $10^6/\mu$l	33.38 g/dl
Std. Error of Mean		.98767	.27065	.1965 8	.09695	.43578
Median		60.8000	20.6000	9.950 0	5.0400	33.9000
Mode		56.80	21.00	9.80	4.66	31.80
Standard Deviation		3.13	0.814	0.490	0.270	1.772
Minimum		52.70	18.10	8.40	4.51	29.80
Maximum		67.10	22.00	11.50	6.03	35.90

Table 3: A comparative analysis of CBC parameters in BTT individuals with previous studies

S.No.	Hematological Parameters	This study	Border et al., 2015[31]	Arora et al., 2017[32]	Parthasarath, 2012 (Mean)[33]	Jameel et al., 2015[34]
1	Hemoglobin g/dl	10.13 0.490	11.7 1.59	9.05 0.05	10.9g/dl	7.3 0.8
2	MCV (fl)	60.95 3.13	66.2 5.15	63.37 1.27	62.9	53.2 2.5
3	MCH (pg)	20.30 0.814	20.7 1.57	18.8 1.8	19.4	18.8 1.8
4	MCHC (g/dl)	33.38 1.772	31.2 1.13	28.00 0.00	30.3	35.2 3.0
5	RBCs ($10^6/\mu$l)	5.04 0.27	5.69 0.80	4.98 5.77	5.70	5.81 2.2
6	RDW	17.5 1.57		17.35 1.57	17.6	16.5 1.8

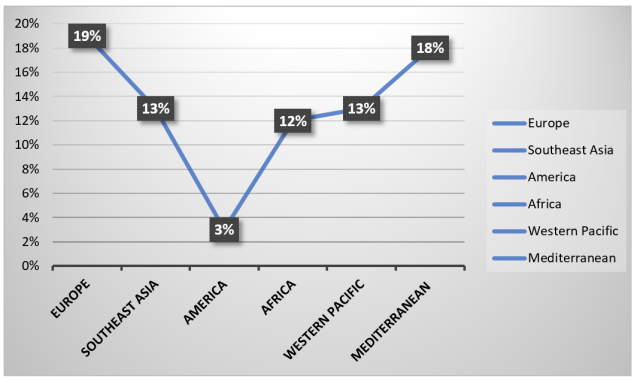

Fig. 1: Prevalence of beta thalassemia trait worldwide

have a high prevalence of this condition. However, we need to focus more on the problem in order to tackle this issue. One of the pre-eminent causes of elevation in BTT cases may be conventional consanguineous marriages and over-population. So, looking for an effective preventive treatment strategy is the ultimate and foremost option to overcome the hazardous outcomes of elevated beta-thalassemia cases in Pakistan.

Moreover, it was also revealed from the study that CBC parameters, including MCV, MCH, RDW-SD, Hb, and RBCs, play an important role in BTT screening. At the same time, RDW and RBCs were proved to be the most dependable parameters to differentiate BTT from IDA.[37] For instance, the RBCs count was higher in BTT as compared to IDA in the selected population. Mentzer's test is one of the reliable tools to screen thalassemia trait cases. According to previous studies, Mentzer's index in thalassemia trait cases is lower than 13 and proved to be a dependable test for the screening of BTT.[38] In the present study, the mean value of Mentzer's test was found to be 12.10. More importantly, the specificity of M.I was found to be 100% indicating the Mentzer's index to be a reliable

test for the detection of BTT for people unable to afford the costly testing.

Furthermore, previous studies have also focused on the mutations being more common in BTT patients. In our study, too, it was revealed that IVS-1-5 (G-C), F.SC 8/9 (+G) and CD/30 mutations appear to be more common ones. However, commonly reported mutations, including 619bp deletion, CD 41/42, and IVS 1-1, have not been spotted among our studied individuals, which may be primarily due to the small sample size taken. Talking about Pakistan, the most common mutations reported in the Pakistan population include IVS 1-5 (G-C), F.SC 8/9 (+G), CD 41/42 (-TTCT), 619 bp deletion. Other than this, other mutations found in Asian populations, including that of Pakistan, are IVS 1-1 and CD30. Generally speaking, patients suffering from B.T. show mutations more commonly in IVS 1-5 and F.SC 8/9, as is indicated by a study conducted by Ahmed et al. in the charsadda district of Pakistan.[39] Similarly, another study also concluded with IVS 1-5 being the commonest mutation among B.T. patients.[31] Khateeb et al. also revealed the commonest mutations in B.T. patients as IVS 1-5, IVS 1-1, 619 bp deletion, CD8/9, CD 41/42.[32] This indicates that the current study is on par with the previously conducted studies when it comes to the commonest mutations found in B.T. patients. Aside from Pakistan, many studies have been conducted in India on the prevalent mutations in B.T. patients. Among people of Indian origin, the commonest mutations appear to be IVS 1-5 (G-C), CD 8/9(+G), CD 41/42, 619bp deletion, and IVS 1-1. This demonstrates the resemblance between patients from India and Pakistan, which could be attributed to historical migrations of citizens across borders.[32]

The recent study provided us with more in-depth information about the prevalence of thalassemia minor in conceiving females. Apart from that, the study helped to sensitize the community regarding screening of beta thalassemia minor to reduce the incidence of B.T.

5. Conclusion

Genetic counselling and extensive family screening must be executed to decrease the birth rate of thalassemia-affected children. At the same time, awareness among parents and physicians regarding antenatal screening should be expanded in order to reduce the B.T. incidence. A coherent preventive plan must be implemented at the national level to eliminate this life-threatening inherited disorder. Most importantly, molecular screening for novel mutations must be implemented to characterize the epidemiology of B.T. mutations in Pakistan. This will certainly help to control the elevated cases of B.T. and help save millions of lives all over the globe.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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