Screening for Haemoglobinopathies- Better prevent than regret - A Tip of the iceberg experience in a Tertiary Care Hospital in Chennai

Yogalakshmi E, Hemamalini NV*, Chitra S

Department of Pathology, Saveetha medical college and hospital Thandalam, Chennai – 602105, Tamilnadu, India

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ABSTRACT

Hemoglobinopathies are single-gene disorders with autosomal recessive inheritance constituting an important cause of morbidity and mortality, imposing a heavy psychological and economical burden on the affected families. The prevalence of Thalassemia and Haemoglobinopathies had been low previously, but now it is found increasing at a rapid pace, because of the migrant population. Since Our Hospital is located in a unique demographic location, in the outskirts of Chennai, the capital city of Tamil Nadu state, populated by multi-ethnic and multi-linguistic residents of varying socio-economic status, we decided to instigate a random sampling study to unearth the presence of Haemoglobinopathies and Thalassemia syndromes among the patients attending our hospital, for various ailments. Hence a prospective study is conducted to screen the variants of haemoglobin by using the High-Performance Liquid Chromatography (HPLC) technology in Saveetha Medical College Hospital, Chennai, for a period of 18 months from May 2018 to October 2019. Out of 305 patients screened, 119 patients were identified to have Thalassemia traits/hemoglobinopathy. Among the 119 positive patients identified, most of them were unaware of the fact, that they are having a blood related genetic disorder, which could be passed on to their next generation. The major barrier in the implementation of the carrier screening programme, is the lack of knowledge about the genetic transmission of these diseases and the advantages of the carrier screening are preventing the birth of a genetically defective child. Creating awareness about the carrier screening among the reproductive population, becomes the key factor in the reduction of the prevalence of Thalassemia and Haemoglobinopathies. This study discusses an Analytical approach for Screening Thalassemia and Haemoglobinopathies and also insists on a flowchart depicting the action plans to be taken to reduce the prevalence of Thalassemia and Haemoglobinopathies

INTRODUCTION

Haemoglobinopathies are single-gene disorders with autosomal recessive inheritance affecting Red Blood cells and constitute an important cause of morbidity and mortality, imposing a heavy psychological and economical burden on the affected families. Haemoglobinopathies either, affect the structure and functions of RBC’s, or it affects the production of Haemoglobin by the Red Blood cells, resulting in qualitative or quantitative abnormality. Haemoglobinopathies fall into two main groups:
Thalassemia syndromes and structural Hb variants. α - Thalassemia and β - Thalassemia are the main types of Thalassemia syndromes and Sickle cell anaemia, HbC and HbE are the predominant abnormal variants of Haemoglobin, identified worldwide.

**Global Prevalence of Thalassemia**

WHO estimates that 5% of the world’s population (420 million) are carriers of Haemoglobinopathies (Modell and Darlison, 2008) and 300,000 to 400,000 children are born annually with clinically significant haemoglobin disorders (Williams and Weatherall, 2012). Among them around 56,000 conceptions are born with Thalassemia disorders and 30,000 of them would be β - Thalassemia, the majority of them being born in developing countries. (Colah et al., 2017). β - Thalassemia (Thalassemia major), HbE and Sickle cell disease are the major types of Haemoglobinopathies prevalent in India.

**The enormity of the disease extent in India**

The estimated burden of Haemoglobinopathies in India is about 42 million carriers (Nagar et al, 2015) of β - Thalassemia trait, which is the largest in the world. The average prevalence rate of Beta Thalassemia major, is 3%- 4% among the 1.21 billion Indian populations (Mohanty et al., 2013). In India, 10,000- 15,000 children are found to be born with β - Thalassemia major annually, with higher frequency, noticed in the communities like Sindhis, Punjabis, Gujaratris, Mahars, Kolis, Saraswats, Lohanas and Gauras (1.36 MB – MoHFW), 2018 a draft policy.

HbE is prevalent in northeastern and eastern states of India, with frequencies of HbE carriers ranging between 3% to over 50% (Baruah et al., 2014). However, HbE alone, whether in the homozygous or heterozygous form, does not cause any significant clinical disease. HbS is predominantly seen among the scheduled tribes, scheduled castes and other backward castes, among the central, western and southern states with a carrier frequency ranging from 5% to 35% (Urade, 2013). Co-Inheritance of Haemoglobin variants with β - Thalassemia also seems to be very common in these ethnical groups.

**Aim of the study**

Our Hospital is located in a unique demographic location, in the outskirts of Chennai, the capital city of Tamil Nadu state, populated by multi-ethnic and multi-linguistic residents of varying socio-economic status. We decided to instigate a random sampling study to unearth the presence of Haemoglobinopathies and Thalassemia syndromes among the patients attending our hospital, for various ailments.

**METHODOLOGY**

Patient screening, for the presence of Haemoglobinopathies, was done using the High-Performance Liquid Chromatography (HPLC) technology. This was a prospective study conducted in Saveetha Medical College Hospital, Chennai, for a period of 18 months from May 2018 to October 2019. Out of 305 patients, 119 patients were identified to have Thalassemia traits/hemoglobinopathy. Anaemic patients of both genders, with suggestive clinical history with or without lower Mentzer index, were subjected to screening. These patients presented with complaints of fever for evaluation, Respiratory infections, Preoperative anaesthetic evaluation, and Genitourinary problems. Patients, who had a history of blood transfusion within the past of 4 months, were excluded from the study. After obtaining appropriate consent for diagnostic purposes, from these patients, peripheral blood was collected in EDTA vacutainers. The samples were analyzed in Sysmex (XN 1000) for CBC values and were stored at 4-8 °C. Within a week, they were processed in BIO RAD-D-10 HPLC Analyser for Haemoglobinopathies/Thalassemia syndromes. This instrument uses the cation exchange HPLC technology for quantifying the HbF and HbA2 along with other variants of haemoglobin. The retention time of each peak and the area below the curves, is used for the quantization of the various Haemoglobin fractions. The obtained results were tabulated. Continuous variables were tabulated as mean ± SD and the categorical variables were tabulated as frequency tables. All statistical analysis was performed using SPSS 16.0 software.

The retention time and windows for the reagent cartridge used in the D10 instrument (BIO-RAD), for a screening of the haemoglobin variants is shown in (Table 1)

**RESULTS AND DISCUSSION**

Out of the 305 cases investigated, 119 cases were found to have either Beta Thalassemia trait or haemoglobin variants. Out of the 119 cases, 73 cases were found to have Beta Thalassemia Traits, 6 cases were found to have Sickle cell anaemia (homozygous), 12 cases were found to have Sickle cell trait (heterozygous), 5 cases were Hb D Punjab trait and Hb E anaemia (homozygous) were found to be 18 cases and Hb E trait (heterozygous) were 5 cases and the distribution of variants according to our study is shown in (Table 2).
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Table 1: Retention time windows, for the reagent cartridge used in the D10 instrument (BIO-RAD), for HPLC analysis of the Haemoglobin chain.

| Sl. No | Peak Name    | Retention time (minutes) | Window (Minutes) |
|--------|--------------|--------------------------|------------------|
| 1      | A1a          | 0.21                     | 0.16-0.26        |
| 2      | A1b          | 0.3                      | 0.24-0.36        |
| 3      | F            | 0.48                     | 0.38-0.58        |
| 4      | LA1c/CHb-1   | 0.755                    | 0.58-0.93        |
| 5      | LA1c/CHb-2   | 0.795                    | 0.66-0.93        |
| 6      | A1c          | 0.87                     | 0.70-1.04        |
| 7      | P3           | 1.43                     | 1.23-1.63        |
| 8      | A0           | 1.7                      | 1.55-1.85        |
| 9      | A2           | 3.15                     | 2.80-3.50        |
| 10     | S            | 4.16                     | 4.02-4.30        |
| 11     | C            | 4.75                     | 4.55-4.85        |

Table 2: Distribution of Thalassemia, Sickle cell Disease and Hb variants, according to our study

| Sl. NO | Identified variants (n=119) | Total No. of variants (n=119) | Frequency distribution |
|--------|-----------------------------|-------------------------------|------------------------|
| 1      | Beta Thalassemia trait      | 73                            | 62%                    |
| 2      | Sickle cell trait           | 12                            | 10%                    |
| 3      | Sickle cell anaemia         | 6                             | 5%                     |
| 3      | Haemoglobin D trait         | 5                             | 4%                     |
| 4      | Haemoglobin E anaemia       | 18                            | 15%                    |
| 5      | Haemoglobin E trait         | 5                             | 4%                     |
| 7      | Total                       | 119                           |                        |

The geographical distributions of variants of haemoglobins according to our study is shown in (Figure 1)

Out of 119 cases, most of the patients (24 cases), were identified to have an ethnic origin from Assam, and the next predominant areas, with affected patients identified, were from Tamil Nadu (Thiruvallur 24 cases & Kanchipuram district 21 cases). Following that, other patients were found to have an origin from West Bengal (11) and Orissa (13).

Out of 73 cases of Beta-Thalassemia trait, 55 cases were Male and 18 cases were females. Sickle cell anaemia was found in 6 males, sickle cell traits were identified in 8 males and 4 females. Hb D Punjab was identified in 5 cases and HbE was identified in 18 males and 5 females.

The Mean, standard deviation and range of Haemoglobin, MCV, MCH, RDW, RBC count, HbA0%, HbA2% and Hb F% are presented in (Table 3). In patients identified with Beta-Thalassemia trait, the mean and S.D of HbA2% was found to be 5.20±0.55, range of (4.0-6.6) and reduced MCV of (62.7±5.8), with a range of (49.4-81.9) and MCH of 19.6±2.22, with range of (14.4-22.7) and increased RBC count 5.98±0.92, range of (2.72 – 8.8) and RDW-CV%. Of 17.8±2.35, with a range of (12.9 - 23.2). In cases of sickle cell anaemia the mean of HbS levels was 69±4.04 and sickle cell trait was found to be 28.3±5.8. Hb D, the mean and SD of HbA2 levels was 2.66±0.44 and HbE anaemia the variant levels of mean and SD was 84.7±4.99 and in HbE trait the variant levels of mean and SD was 19.7-30.5 (Table 3).

Numerous studies have been done in the past, to establish the sensitivity and specificity of HPLC in the diagnosis of Thalassemia and Haemoglobinopathies. The automation and accurate quantisation of various haemoglobin fractions, has made HPLC as a reproducible alternative to Hb electrophoresis. Multiple studies have, (Joutovsky et al., 2004; Warghade et al., 2018; Mondal and Mandal, 2016) stated the precision of the retention times, obtained in the normal and abnormal fractions of haemoglobin, emphasizing the role of HPLC in diagnosing Haemoglobinopathies. The prevalence of Thalassemia and Haemoglobinopathies had
Table 3: Haematological parameters and Haemoglobin profile obtained in HPLC for various types of Thalassemia traits and Haemoglobinopathies expressed as Mean±SD.

| Parameters               | Beta Thal trait (n=7) | Sickle cell anaemia (n=6) | Sickle cell trait (n=12) | HbD Punjab trait (n=5) | HbE Anaemia (18) | HbE trait (5) |
|--------------------------|-----------------------|---------------------------|--------------------------|------------------------|------------------|---------------|
| Mean±SD (Range)          | Hb(gm/dl) 11.7±2.1 (6.9-17.0) | 6.0±2.7 (2.9-9.2) | 12.2±2.2 (7.2-15) | 12.9±1.3 (11.4-14.8) | 11.8±1.6 (7.7-14.6) | 12.1±1.54 (10.3-14.2) |
|                          | MCV(fl) 62.7±5.8 (49.4-81.9) | 73.3±9 (60.8-82.7) | 72.1±10.1 (58.3-89.8) | 75.±7.14 (67.2-84.4) | 59.39±3.5 (53.7-66.6) | 77.8±11.33 (64.5-95.4) |
|                          | MCH(pg) 19.6±2.2 (21.2-33.6) | 25.2±3.4 (30.5-37.7) | 32±1.9 (28.6-34.7) | 25±2.54 (23.1-26.9) | 20.03±1.46 (18.4-23.1) | 25.7±4.34 (23.8-26.6) |
|                          | RBC count 5.9±0.9 (2.72-8.8) | 2.3±0.8 (1.2-3.1) | 5.3±1.1 (3.19-7.1) | 5.2±0.63 (4.5-6.1) | 5.91±0.79 (4.1-7.31) | 5.63±2.28 (3.5-9.5) |
|                          | RDW-CV 17.8±2.3 (12.9-23.2) | 19.0±3.1 (13.8-23.5) | 16.6±1.8 (14.6-18.6) | 14.42±1.92 (12.5-17.6) | 17.5±1.5 (13.2-19.7) | 15.0±0.79 (14.3-16.4) |
|                          | HbA0 (%) 82±2.3 (68.5-85) | 3.1±0.4 (2.6-3.8) | 57.7±5.1 (50.8-52) | 49.94±3.85 (44.1-54.4) | 6.98±2 (4.4-12.8) | 61.2±4.67 (56.2-66.8) |
|                          | HbA2 (%) 5.2±0.5 (4.0-6.6) | 2.5±0.6 (1.9-3.4) | 4.0±9 (3-6.6) | 2.6±0.4 (2.2-3.2) | - | - |
|                          | HbF (%) 1±1.4 (0.8-15.7) | 21.6±3.5 (17.4-26.5) | 1.6±0.5 (0.8-2.2) | 1.1±0.14 (0.8-1.1) | 3.7±2.16 (1.5-9.2) | 1.0±0.1 (0.8-1.2) |
|                          | Variant (%) - | 69±4.0 (64.2-75.4) | 28.3±5.8 (20.1-37) | 33.7±4.6 (25.9-38) | 84.7±4.9 (73.6-90.9) | 26.6±5.4 (19.7-30.5) |

Figure 1: Geographical distributions of beta Thal trait/Haemoglobinopathies according to our study
been low previously, but now it is found increasing at a rapid pace, because of the migrant population (Weatherall and Clegg, 2001) and the rise of intercaste and interreligious marriages.

**Diagnosis of Thalassemia and Haemoglobinopathies**

The diagnosis of Thalassemia and Haemoglobinopathies begins with clinical suspicion in anaemia patients, based on the phenotype, family history, and Laboratory results like Complete Blood Count values, the Mentzer index, peripheral smear studies, CE HPLC, electrophoresis, mass spectrometry and molecular characterisation, using the peripheral blood of the patient. Diagnosis of Thalassemia and hemoglobinopathy in the prenatal period is done by performing chorionic villous sampling in the first trimester of pregnancy and amniocentesis or cordocentesis in the second trimester of pregnancy. Although the analysis of foetal haemoglobin types is successfully performed by HPLC, it is prone to errors due to contamination by maternal tissue. Universal screening programs aimed at detecting carriers and offering prenatal diagnosis in pregnancies at risk for Thalassemia have been adopted in Canada and European countries (Lee et al., 2019; Cousens et al., 2010)

**Anticipated expenditure in Thalassemia and Haemoglobinopathies treatment**

The treatment depends upon the type, severity and complications of Thalassemia and Haemoglobinopathies. The mainstay of treatment is frequent blood transfusions, Iron chelation and Bone marrow and stem cell transplant. Blood transfusions in Thalassemia will have to be given every 3 to 4 weeks, depending upon the severity. Patients have to undergo frequent laboratory investigations like complete blood count, Iron profile, renal function test, liver function test and cardiac profile. Management of complications of transfusion like iron overload, transmissible transfusion infections, osteoporosis and endocrine dysfunctions also becomes an added hindrance to the survival of the patients. Only a small percentage of patients are fortunate enough to undergo bone marrow transplant as a permanent solution for this condition. The majority of patients suffering from Thalassemia are unable to get a permanent solution due to a lack of infrastructure and financial support due to the prohibitive cost involved.

According to WHO, the estimated expenditure involved in the treatment of Thalassemia according to the regular standards is around US$ 8658 worth medications and 27 units of packed Red blood suspensions, per annum. A recent Indian study states that the estimated average expenditure annually for a child in our country, is around Rs. 1, 00,000 to Rs. 2, 50,000/- depending upon the age and the presence of complications. The cost of Bone marrow transplant is estimated to be Rs.14...
Figure 3: Flowchart depicting the action plans to be taken to reduce the prevalence of Thalassemia and Haemoglobinopathies
lakhs to 15 lakhs approximately. With more and more patients being brought under the care by transfusion and chelation, the requirement for blood transfusions has increased exponentially. It is estimated that globally, 2 million units of packed RBC bags are required for treating the Thalassemia patients annually. Allogenic stem cell transplant remains the mainstay of management, which is successful in only 90% of patients. A recent study states that, the life expectancy of a Thalassemic patient, in spite of treatment, is 35.6±7.4 years. Besides the financial burden, the psychological stress to which, the family and the affected child is imposed to, becomes unbearable. Thus, it would be practical to consider that, the premarital carrier state screening, genetic counseling and prenatal diagnosis of Thalassemia and Haemoglobinopathies are, as important as the diagnosis and treatment of the above conditions (Ryan et al., 2010).

An analytical approach for Screening Thalassemia and Haemoglobinopathies

RBC indices and HPLC analysis for haemoglobins are useful and cheaper options for the screening of Thalassemia carriers and Haemoglobin variants. The parameters like MCV < 80fl, MCH <27pg, Total RBC count (High, normal or low) and RDW (raised or normal), obtained from the Complete blood count and Hb A2 cut off value below 3.5%, from the HPLC analysis are used for screening purposes. The algorithm below shown in Figure 2 illustrates the carrier status of an individual based on the variations identified in the RBC indices and the HPLC analysis. (Figure 2)

As Iron deficiency anaemia is also common, in our country, a primary care physician must be able to differentiate it from a B Thalassemia trait. Both the patient will present with reduced Hb values with microcytosis, hypochromia, reduced MCV and reduced MCH values. But in IDA, the patient will have a consequent reduction in the RBC count with a proportionate increase in the value of RDW and normal HbA2 values, in contrast to an increase in the RBC count, normal RDW and reduced HbA2 values in a Thalassemic patient. A diagnostic dilemma is experienced when the Hb A2 value is near normal/borderline (3.0%-3.9%). In India, borderline HbA2 with near-normal or reduced red cell indices in β Thalassemia heterozygote is predominantly seen, due to the campsite b1(A > C) mutation and the poly-A (T > C) mutation but β Thalassemia traits with other mutations who also show borderline or normal HbA2 levels do co-exist. Borderline HbA2 levels are also identified in patients who are on Zidovudine and stavudine, by which they can get misdiagnoses as Thalassemia carriers (Bhagat et al., 2016). Based on the retention time, in the HPLC, other haemoglobin variants also be identified. This person’s carrier status can be identified using the RBC indices and HPLC report.

Thalassemia carrier screening Programmes - Plan of approach

Β-Thalassemia (heterozygote), carrier screening programmes are available, throughout the world. The majority of Thalassemia carriers have a reduced Mean corpuscular volume and Mean corpuscular haemoglobin values in a complete blood count. In 1998, the World Health Organisation, published that no genetic testing should be carried out compulsorily. Nevertheless, the premarital B-Thalassemia carrier screening programmes are conducted worldwide, screening the young population in the reproductive age group, after obtaining informed consent from them. Carrier screening of B-Thalassemia was started in Cyprus as early as 1973, where the rate of carrier status of B Thalassemia is highest, as high as one in seven-person. Other countries, where the carrier screening programmes are conducted are Greece, Italy, France, Israel, England, Maldives, Taiwan, China, Iran, Palestine and Turkey and Canada (Giordano et al., 2014). Numerous research work has already been done in this field, that the prevalence of B-Thalassemia and Haemoglobinopathies have reduced, following the implementation of premarital carrier screening programmes followed by genetic counseling for the couples, in the event of one of the partner is either a Thalassemia or hemoglobinopathy carrier.

The major barrier in the implementation of the carrier screening programme, is the lack of knowledge about the genetic transmission of these diseases and the advantages of the carrier screening in preventing the birth of a genetically defective child. Creating awareness about the carrier screening among the reproductive population, (Henneman et al., 2016) becomes the key factor in the reduction of the prevalence of Thalassemia and Haemoglobinopathies, in the long run.

The flow chart (Figure 3), represents the plan of approach, right from creating awareness about Thalassemia and Haemoglobinopathies among the younger population and early screening methods in the premarital periods, fetal diagnosis in the prenatal period.

CONCLUSION

The data obtained from our study, for the screening of Thalassemia and Haemoglobin variants...
shows that, CE HPLC as an excellent, cost-effective methodology for mass screening and consequent reduction in the incidence of Thalassemia and Haemoglobinopathies. Our study recorded about 119 cases with Beta-Thalassemia traits and Haemoglobinopathies, as an incidental finding, among 305 cases screened. Out of 119 positive patients identified, most of them were unaware of the fact, that they are suffering from a blood related genetic disorder, which could be passed on to their next generation. The high prevalence of Beta Thalassemia trait in India makes the screening programs for the above diseases, as a mandatory entity and CE HPLC, has become an inevitable methodology, for rapid and definitive diagnosis. Screening and prevention of the birth of a fetus implicated with either BTT or Haemoglobinopathies appear to be more effectual, rather than, to endure the psychosocial and economic burden, imposed on the family while treating the affected child. A joint effort by the State Government, with NGO aids and Thalassemia societies, should be strategized in a systematic manner for the successful implementation of a nationwide control of Thalassemia and other Haemoglobinopathies.

Conflict of interests
No conflicts of interest.

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