Research Paper

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Retrograde Study of Screening of Medical & Paramedical Students of Ahmedabad Municipal Corporation For Beta Thalassemia Trait

Medical Science

KEYWORDS : Screening, Beta thalassemia trait, AMC medical & paramedical students

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ABSTRACT

INTRODUCTION

Haemoglobinopathies are common genetic disorders of haemoglobin in which there is abnormal production or structure of haemoglobin molecule. These genetic disorders are major public health problem in many parts of the world including India. Population screening and genetic counselling are very important for prevention of thalassemia major & other hemoglobinopathies.

AIMS & OBJECTIVES

To know the incidence of beta thalassemia trait of AMC medical & paramedical college students.

To find out the advantages of HPLC & other haematological tests for diagnosis of hemoglobinopathies and correlate them.

To acknowledge for prevention of thalassemia major by identifying beta thalassemia trait in targeted population.

MATERIAL & MATHODS

A total 1888 students were analysed in the months of September to November 2012 for screening of beta thalassemia trait. All blood samples were tested on Cell Dyn 3700 to derive values of Hb, RBC count & Red blood cell indices. The screening precision was based on Menzer's ratio< 13 (MCV/RBC count). These samples were further evaluated for peripheral blood smear examination, Hb electrophoresis on Cellular Acetate Agarose Gel (Ph 8.9), HbA2 estimation by HPLC on BIORAD VARIANT II for final conclusion.

RESULTS:

On analysis of 1888 cases, 132 cases showed Menzer's ratio < 13. These samples were evaluated for peripheral blood examination & Hb electrophoresis. For HbA2 quantitation, samples were tested on HPLC. A cut off value of HbA2 > 3.5% was considered for diagnosis of beta thalassemia trait. HPLC results showed 40 cases (2.11%) of thalassemia trait, HPLC result also showed other hemoglobinopathies.

CONCLUSION:

Prospective prevention through population screening is one of the best possible strategy for prevention of thalassemia major. HPLC is accurate & reliable technique with combination of other hematological tests which makes them an ideal method for screening purpose.

INTRODUCTION:

'Prevention is better than cure', holds true for all diseases but is especially true for most genetic disorders where prevention is still the only option. Awareness and education are prerequisites for any screening programme for genetic diseases[1]. Beta thalassemia major is a serious and major cause of morbidity. Availability of reliable and sensitive screening test is an important advantage for prevention of thalassemia major and other hemoglobinopathies i.e. sickle cell anemia.

Screening of hemoglobin disorders is usually discussed with respect to two target population: neonates and adults of reproductive age. Significance of screening of hemoglobinopathies is useful for preventive measures, to avoid incorrect diagnosis & to determine trait status.

Thalassemia is the most common type of hemoglobinopathy. These disorders are the results of the existence of an abnormal allele, in one or more globin genes. The decrease or loss of an α or β chain, as two types of thalassemia has unfavourable effects on the production and survival of red blood cells and may cause a decrease in the concentration of the globin chain and hemoglobin, resulting in microcytosis and hypochromia.[2]

Beta thalassemia is the most common in India with overall prevalence of 3 - 4%. In certain communities like Sindhis, Muslims, Cutchi, Bhanushalis & some tribal groups, the prevalence of beta thalassemia carries between 8 & 10% or more.[3] WHO figures estimate that 5% of the world population is carrier for Hb disorder.[4]

MATERIAL & METHODS:

The screening programme of beta thalassemia trait for students of AMC medical, dental, physiotherapy & nursing colleges was conducted in the months of September to November 2012. Total 1888 students were analysed.

All the students filled & signed the consent forms which included information regarding their name, age, sex, caste, address, family history & past history. From each student blood samples were collected in two separate EDTA tubes by experienced phlebotomist.

All the samples were tested on automated cell counter (CELL DYN 3700 - Abott). Value of Hb, RBC count, RBC indices & RDW were noted. Menzer's ratio (MCV/RBC count) was calculated from all the samples. A cut off value for MR<13 was considered. On samples having Menzer's ratio<13, peripheral smear examination, Hb electrophoresis & HbA2 quantitation were performed. Hb electrophoresis was done on cellulose acetate agarose gel at PH 8.9. HbA2 quantitation was done on BIORAD VARIANT II which utilizes the principle of high performance liquid chromatography (HPLC).

From above data, final results were prepared.

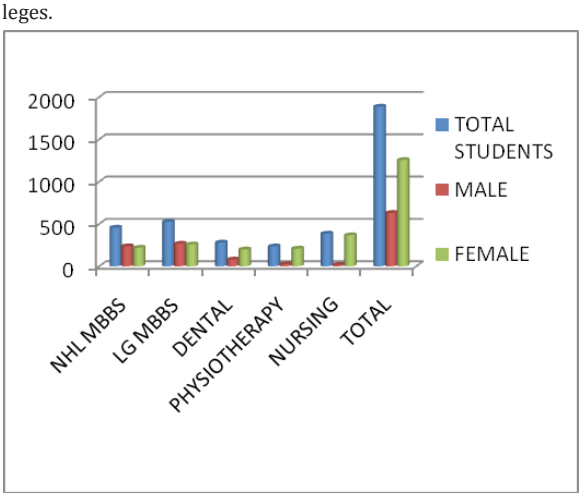
RESULTS:
The study attempted to find out beta thalassemia trait in 1888 students.

TABLE I

NAME OF COLLEGE	NUMBER OF STUDENTS	MALE	FEMALE
NHL MBBS	457	237	220
LG MBBS	527	268	259
DENTAL	281	81	200
PHYSIOTHERAPY	236	26	210
NURSING	387	19	368
TOTAL	1888	631	1257

Out of total 1888 students, 631 were males and 1257 were females with M: F was 1:2.

Females were almost double in number than males because of more number of girls in dental, physiotherapy and nursing colleges.



All blood samples were run on CELL DYN 3700 analyser. Out of 1888 samples analysed, 132 showed Menzer's ratio<13, RBC count ≥ 5 million/cu mm with MCV < 70 fL & MCH < 22pg. Peripheral smear examination of these samples showed hypochromic microcytic RBCS with target cells in few cases..

Hb electrophoresis of 40 samples showed thick band of HbA and thin band of HbA2. 11 cases showed thick band of HbA and thin band of HbS. In one case, bands of HbA, F and S were seen. In one case bands of HbA & F were seen. 79 cases showed normal electrophoresis.

For final confirmation of 132 cases, samples were run on HPLC BIORAD VARIANT II.

TABLE II (Result of HPLC)

COLLEGE	Number of Students	Menzer's Ratio	β – thal trait	Other hemoglobinopathies				Normal
				α–thal trait	Sickle cell trait	S/β^+ thal	HPFH	
VSGH	457	22	09	04	03	-	-	6
LG	527	43	11	18	03	-	01	10
DENTAL	281	11	07	01	01	-	-	2
PHYSIOTHERAPY	236	12	03	04	03	-	-	2
NURSING	387	44	10	05	01	01	-	27
TOTAL	1888	132	40	32	11	01	01	47

Out of 132 cases, 40 cases showed beta thalassemia trait with HbA2>3.5%., 45 cases of other hemoglobinopathies in which 32 cases of possibility of α thalassemia, 11 cases of sickle cell trait, one case of sickle/ β^+ thalassemia and one case of HPFH. 47 cases showed normal HPLC result.

DISCUSSION
There are two main groups of inherited disorders of hemoglobin: 1) Structural variants with defects in structure and 2) Thalassemias with defects in the synthesis of globin chain. An overlap has been noted between these two groups. Most thalassemias involve α or β globin chains. Hemoglobinopathies can be homozygous or heterozygous.[5]

Thalassemia is a major public health problem in India.[6] The carrier rates range from 2 – 19% in the different population.[7] In view of high prevalence of hemoglobinopathies, a routine pre marital screening programme is needed for identification & prevention of high risk marriages.[8]

Beta thalassemia trait is caused by the β° - thalassemia gene with absence or β^+ - thalassemia gene with reduced, beta globin chain synthesis. There are usually no symptoms or abnormal physical sign. Most β- thalassemia heterozygous have a mild anemia, RBC count is elevated (5 – 7 M/μL) with low MCH (<22 pg), & MCV (between 55 and 70 fL) on stained films, the cells have a moderate degree of microcytosis, hypochromia, anisocytosis and poikilocytosis with few smears showing target cells & basophilic stippling.[5]

In our study out of 1888 cases, 132 cases had Menzer's ratio <13 (MCV/ RBC count). The diagnosis of beta thalassemia trait was verified by Hb electrophoresis at pH 8.9 and quantitation of HbA_2 fraction by HPLC.

HPLC is a method of choice, it provides accurate measurement of HbF, along with separation of Hb variants. HPLC offers distinct advantage over classic Hb electrophoresis as it can more accurately identify and quantitate abnormal hemoglobin [9]

HPLC is an excellent, powerful diagnostic tool for the direct identification of haemoglobin variants with a high degree of precision in the quantification of major and minor, normal and abnormal haemoglobin fractions.[10]

HPLC reports suggested 40 cases (2.11%) of beta thalassemia trait with HbA_2 level >3.5%. Hb electrophoresis showed thick band of HbA & thin band of HbA_2 32 cases (1.69%) were reported as possibility of α – thalassemia on HPLC with normal HbA_2 & HbF quantitation. Hb electrophoresis was normal.11 cases (0.58%) were diagnosed as sickle cell trait on HPLC having 'S' window ranging from 20- 40%. Hb electrophoresis showed presence of thick band of HbA & thin band of HbS. Sickling test was positive. One case was diagnosed as possibility of HPFH (HbF – 20%, HbA_2 – 2.6%) on HPLC. Electrophoresis showed band of HbA & band of HbF. One case was double heterozygous for sickle/β^+ thalassemia on HPLC confirmed by band of HbA, HbS & HbF on electrophoresis with positive sickling test. Parental study was also helpful in this case.

TABLE III
Comparison of prevalence of beta thalassemia trait among medical students.[11,12,13]

Name of study	Percentage of medical students having thalassemia (%)
Paresh et al (2006) B.J. Medical college, Ahmedabad	1.6%
Sumera et al (2011) Dow Medical college, Karachi	5.3%
Wahidiyat et al (2011) Eijtman Institute, Indonesia	4.76%
Our study (2012) AMC Medical & Paramedical Students, Ahmedabad	2.11%

The prevalence of beta thalassemia trait in our study is comparable with Paresh et al study.

As discussed above, amongst 132 cases, 85 cases were positive for hemoglobinopathies. Remaining 47 cases showed normal HPLC & Hb electrophoresis results, were affected by other causes of microcytosis & hypochromia.

The differential diagnosis between iron deficiency & beta thalassemia or α – thalassemia trait can be difficult. Increased RBC count in presence of decreased MCV is the hallmark of thalassemia trait.[14] RBC indices like low MCH, MCV, RBC count with Menzer's ratio>13 & increased RDW are favouring diagnosis of iron deficiency anemia but this & other formulas are not conclusive enough for diagnosis. HbA_2 & HbF quantitation by HPLC & serum ferritin level are useful to exclude iron deficiency anemia.[14,15] For better interpretation of HPLC results, HbA_2 quantitation should be done after correction of iron deficiency anemia.

Limitation of HPLC technique is its higher cost and expertization for interpreting the results. Interpretation of HPLC results like possibility of α – thalassemia & other hemoglobinopathies that elute with similar retention values cannot be ruled out by HPLC. Therefore hemogram findings, Hb electrophoresis, & HPLC are added with molecular studies like linear Array, Gap – PCR & DNA Sequencing to conclude the final diagnosis.[16]

SUMMARY AND CONCLUSION

A major outcome of beta thalassemia carrier screening is a reduction in the incidence of beta thalassemia major which is serious & major cause of morbidity.[17] Premarital screening is acceptable & the most effective strategy for control of thalassemia in developing countries like India.[18]

To conclude hemogram by automation, Hb electrophoresis & HPLC are ideal methods for routine screening of beta thalassemia trait.

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