

Screening to Determine Prevalence of β -Thalassemia and Iron Deficiency Anemia Among Medical Students

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Abstract: According to the Thalassemia Federation of Pakistan, the mostly inherited disorder in Pakistan is β -thalassemia, which is characterized by a deficient, abnormal, or lack of β -globin chain synthesis and has a prevalence of 6%. The only method of controlling and preventing β -thalassemia is to increase awareness among students. This was an observational study using a random sampling technique. The Dow-Thalassemia awareness program recruited 915 medical students from the Dow Medical College (DMC) and Sindh Medical College (SMC) to voluntarily donate blood samples, which were analyzed by the naked eye single tube red cell osmotic fragility test (NESTROFT) and complete blood count and results were confirmed by high-performance liquid chromatography and analyzed using the NESTROFT. The samples were collected in 2012-2013. A total of 915 samples, out of these 390 samples, 390/915 (42.6%) samples were positive and complete blood count found 282 (72.3%) were positive for iron deficiency anemia. The remaining 108/390 (27.6%) were confirmed by high-performance liquid chromatography. Only 2.4 % subjects were positive for the β -thalassemia trait. Of 915 students, 57.4% of students were healthy, 39.2% had iron deficiency anemia, and 2.4% were carriers of the β -thalassemia trait. The overall prevalence of β -thalassemia was 38/915 (4.1%), which was lower than observed in previous studies. This study also demonstrated the NESTROFT can be used as a primary method of screening out healthy individuals, where approximately 50% require further screening for β -thalassemia.

Keywords: Thalassemia minor, NESTROFT, HPLC, Pakistan, β -thalassemia trait, IDA

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Introduction:

β -thalassemia is a blood disorder involving defects in functional β -globin chain synthesis. Abnormalities in this globin chain result in diverse phenotypes that range from being clinically asymptomatic to severe anemia. This disorder affects 5% of the world population, where 5,000 babies are born with thalassemia in Pakistan annually, 5 out of 100 people are thalassaemic, and around 8 million are carriers for thalassemia[1]. Previously, [2] reported there are approximately 9.8 million carriers in Pakistan, making up 5-7% of the total Pakistani population. Today, this population has increased to over 200 million people, making it the world's sixth-most populous country[3]. To prevent thalassemia, 46 associations in Pakistan formed the Thalassemia Federation of Pakistan.

The most common nutritional deficiency worldwide is anemia, which is associated with an iron-poor diet in 95% of cases. One of the factors contributing to this problem is the large number of people living in underdeveloped countries where nutritional deficiencies are common. Researchers have reported on the mental and physical health problems associated with poor dietary intake[4]. Iron deficiency anemia (IDA) is a hematological condition defined as a decreased hemoglobin concentration and red blood cell (RBC) count with respect to gender and age. The prevalence of iron deficiency in the healthy teenage population is an important indicator of the general nutritional status of the population. In 2002, the World Health Organization estimated two billion people are anemic worldwide with IDA being the most common form[5]. The aim of the study to check whether NESTROFT can be used as a primary screening technique in remote and underdeveloped areas in the country.

The Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) is used for mass screening and field trials for anemia due to being inexpensive and easy to perform. To diagnose β -thalassemia, the HbA2 concentration is measured after RBC lysis by high-performance liquid chromatography (HPLC) with electrophoresis or ion-exchange[6].

This is considered the "gold standard" and is highly sensitive and specific. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are used for the preliminary selection of individuals who are at risk of being heterozygous for thalassemia, where MCVs ≤ 80 fL and MCHs < 27 pg are indicative of thalassemia. All β -thalassaemic individuals have increased HbA2 concentrations[7].

Another form of thalassemia is known as silent β -thalassemia, where individuals with β -thalassemia have particularly low levels of β -globin chains[8]. For hemoglobin electrophoresis, heterozygous individuals have almost normal or minimally reduced RBCs and normal HbA2 levels and, therefore, are difficult to identify by conventional methods[9].



These carriers need to be evaluated using molecular studies. For example, in the Mediterranean region, a -101(C-T) mutation has been reported, while a CAP+1(A-C) mutation has been documented in Pakistan and other South Asian regions[9]. The most prevalent mutation among all ethnic groups in Pakistan has been reported by [10] as Intervening Sequence 1-5 (G-C), where 40.89% of carriers in Pakistan have this mutation. Molecular analysis revealed cases of β -thalassemia are very heterogeneous and there have been reports of more than 200 associated point mutations and deletions of different severities[9,10]. The only way to prevent and control IDA and β -thalassemia is to create awareness among teenagers. Karachi is considered the largest cosmopolitan city of Pakistan with a population of 23.7 million people[11]. This present study was conducted in two medical colleges in Karachi. Because these medical colleges are considered the best in the country, the student population originates from around the country and is representative of almost all ethnic groups.

2. MATERIALS AND METHODS

This present study was undertaken in two different hospitals in Karachi, Pakistan in 2012-2013. A total of 915 blood samples from both genders from the normal student population was collected in tubes containing ethylene diamine tetraacetic acid (EDTA). Participation in this study was optional and informed consent was obtained from each enrolled student. The participants were recruited under TAP-DOW (Thalassemia Awareness Program, Dow University) and approved by the Institute.

The participants were enrolled as per guidelines of the Helsinki Declaration 1975, self-autonomy, confidentiality and rights to withdraw were given to the subjects.

All 915 blood samples were screened for the β -thalassemia trait using the NESTROFT [12,13]. The results of the NESTROFT were confirmed by a Complete Blood Count (CBC), which measured the erythrocyte parameters of MCV, MCH, RBC, red cell distribution width, and hemoglobin, performed using an automated hematology analyzer (Sysmex NE1500). Hemoglobin electrophoresis was used, where carriers of the β -thalassemia trait subjects had HbA2 levels >3.5% and controls had normal hematological and HbA2 values. HPLC was performed using an automated HPLC system (D-10 Bio-Rad). The frequencies and data analysis were conducted on Microsoft Excel.

3. RESULTS AND DISCUSSION

A total of 915 medical students aged 18-22 years voluntarily provided blood samples. Of these students, 35.8% were male and 64.2% were female (Fig. 1). All collected blood samples were analyzed by NESTROFT and CBC and then confirmed by HPLC.

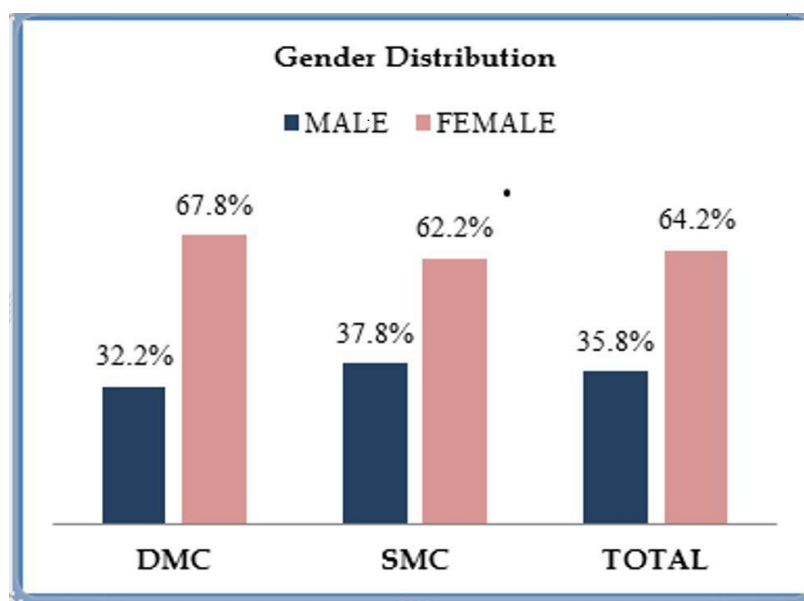


Fig. 1. Gender distribution of cases.

Of the 915 samples, 42.6% were found to be positive by the NESTROFT. The CBC revealed 39.2% of these subjects had IDA. The remaining 27.7% were analyzed by HPLC in order to detect carriage of the β -thalassemia trait. Only 2.4% of all tested subjects were confirmed to carry the β -thalassemia trait (Fig. 2).

The NESTROFT was used to identify cases of IDA and carriers of β -thalassemia trait. There are a number of reasons for abnormal RBC osmotic fragility, which results in altered RBC shape and function. RBC shape can be altered due to defective genes or insufficient production of certain proteins; these RBCs would yield NESTROFT-positive results (Fig. 3).



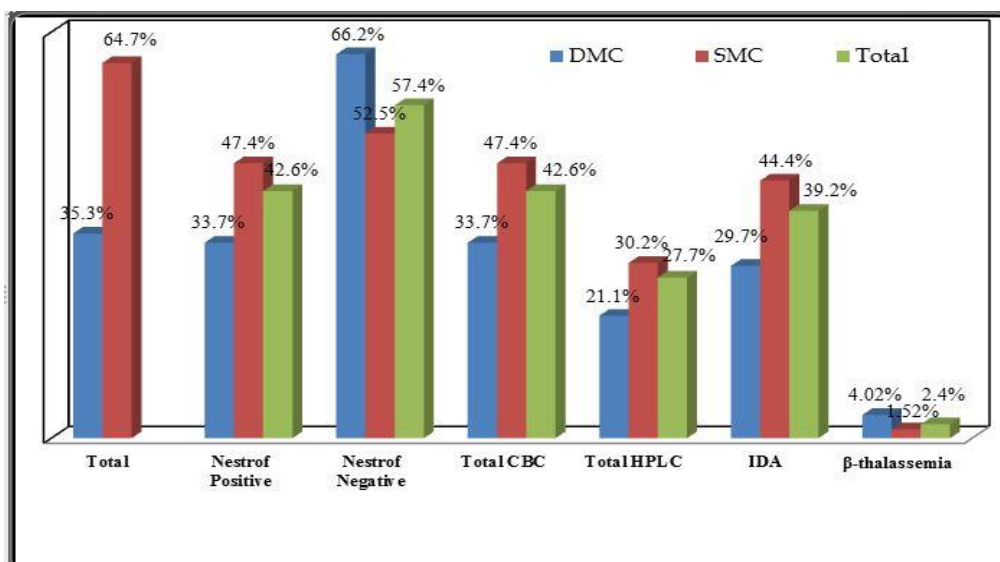


Fig. 2. Diagnostic distribution of the cases

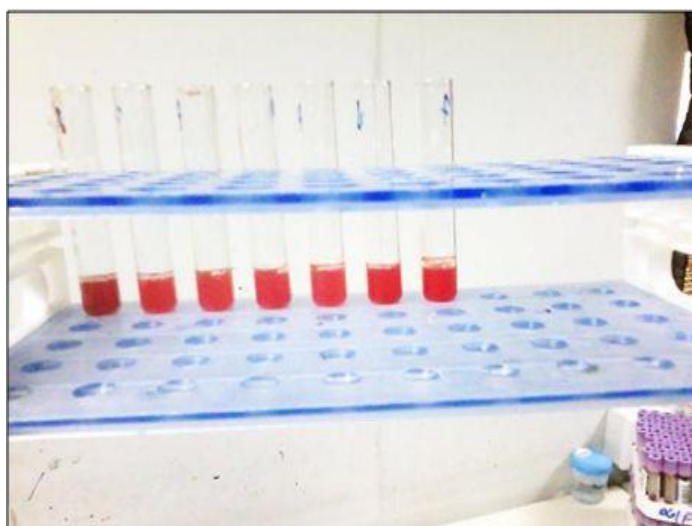


Fig. 3. The naked eye single tube red cell osmotic fragility test (NESTROFT).

In this present study, the NESTROFT aided in initial screening of the population. The sensitivity was found to be 93% in a previous study conducted at the Dow Institute of Hematology [14]. Thomas and et al, has also been published by [15], while Singh et al and Amini et al, reported a sensitivity of more than 95% [13] and [16]. Gorakshakar et al, reported sensitivity ranging from 98 to 100%. Similar results were obtained in a study by Thomas et al, observed NESTROFT sensitivity to be 95.5%. Amini et al, also noted a sensitivity of 100% [16]. In a study of the North Indian Punjabi population, the test displayed a 100% sensitivity [17]. In a study by [18], NESTROFT has an overall sensitivity and specificity of 95 and 95.8%, respectively. Based on these studies and our analysis, a positive NESTROFT test can be used for initial screening for IDA and β -thalassemia trait, where β -thalassemia can be easily excluded in NESTROFT-negative cases. However, it should be noted this test could miss a few cases of thalassemia minor, which can become potential sources of defective genes inherited by future generations.

CBC analysis revealed 39.2% of subjects were positive for IDA. The remaining 27% of NESTROFT-positive subjects were analyzed by HPLC to confirm the presence of the β -thalassemia trait. The erythrocyte indices of MCV, MCH, RBC, red cell distribution width, and hemoglobin were successfully measured (Table 1). The cellular morphology in students with IDA or carrying β -thalassemia trait presented with similar blood parameter values (Figure 4). [19], previously discussed the importance of optimal therapeutic management of IDA in children in whom prevention has failed, but little emphasis has been placed on teenagers. In India, 55% of adolescent girls were found to anemic [20]. About 50% of pregnant woman and 40% of preschool children in developing countries are estimated to be anemic because of malnutrition and, in many cases, an iron-deficient diet [21].



	CBC					HPLC		
	Hb	RBC	MCV	MCH	MCHC	A1	A2	F
Normal Values	12-14	3.8-5.2	80-100	27-32		95.5-98.0	1.5-3.5	< 2.0
β-thalassemia trait	9.8-11.7	5.6-6.0	56-63	17-19	30-32	93.7-93.9	4.9-5.5	0.8-1.4
IDA	8.7-10.7	4.3-5.1	78-58	17-25	29-31	97.6-97.7	1.5-1.6	0.8
Borderline	10.9	5.67	60.5	19	32	95.4	3.5	1.1
Increased HbA2	13.2	4.9	85	27	32	93.0	5.4	1.6
Silent β-thalassemia trait	11.2-12.2	5.49-6.83	6.5-72	20-23	30-32	96.6-97.1	2.3-2.8	0.8

Table1: Erythrocyte indices by complete blood picture (CBC) and HPLC

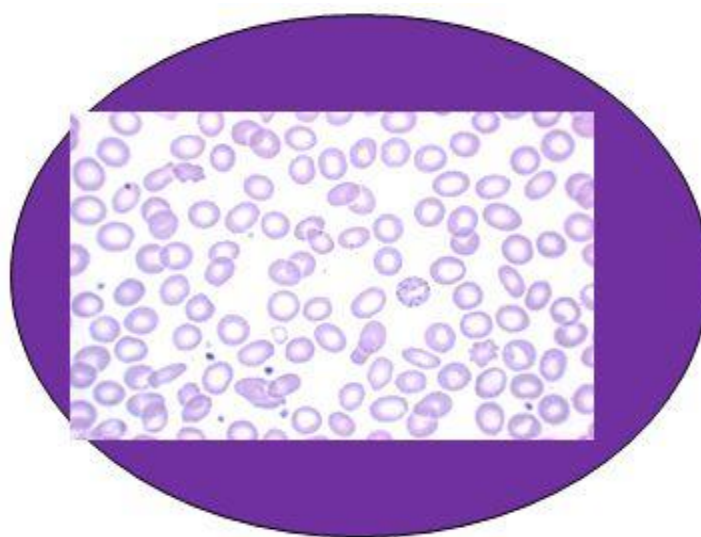


Fig. 4. Cellular morphology of RBC during IDA and β -thalassemia trait.

HPLC results revealed 27.7% of all subjects assessed had IDA, while 2.4% carried β -thalassemia trait. Further analysis of these HPLC results determined 10/23 of β -thalassemia carriers (43.4%) had IDA. Of these 10 carriers of β -thalassemia trait, 4/23 cases (17.3%) were confirmed to carry β -thalassemia trait, 5/23 subjects were silent carriers of β -thalassemia trait (21.3%), and 1/23 (4.3%) was found to be a borderline and unique case (4.3%) as the CBC parameters were normal with a borderline MCH of 27 pg value and increased levels of HbA2 (Table 1). Previously, [22] reported on β -thalassemia with exceptionally high HbA2 levels of 8.4 to 11.2%, where HbA2 levels in individuals heterozygous for β -thalassemia averages about 5% and rarely exceeds 7% (Fig. 5) and in normal adults are rarely greater than 3.5%.

Based on the above results, the prevalence of β -thalassemia trait is 2.4% among medical students in Karachi, Pakistan. Other scientists have also reported on the prevalence of the β -thalassemia trait. [23] reported there are 8 million carriers of thalassemia among the Pakistani population. Previously, [2] estimated a carrier rate of 5-7% with a total of 9.8 million carriers in the Pakistani population. Today, this Pakistani population has increased to over 188 million, making it the world's sixth-most populous country [23] reported the prevalence of β -thalassemia trait averages 2.78%, ranging from 1.48 to 3.64 % in different states in India with a prevalence of 0 to 9.3% β -thalassemia trait across 59 ethnic groups. [24] reported the gene frequency averages 4.05%, with 2.68 and 5.47% of children being carriers in Mumbai and Delhi, respectively. [25] reported Turkey is at high risk of having an increased frequency of the β -thalassemia trait compared to other cities, such as Sanliurfa Province, where the prevalence of β -thalassemia with a concurrent high HbA2 (>3.5%) was 2.44% ($n = 1853$).

Cases of borderline disease based on HPLC, elevated HbA2, and silent β -thalassemia trait should be confirmed using DNA-based tests. Several scientific approaches have HbA2 used to determine the incidence of β -thalassemia and identify (Fig. 6) the prevalence of associated mutations.



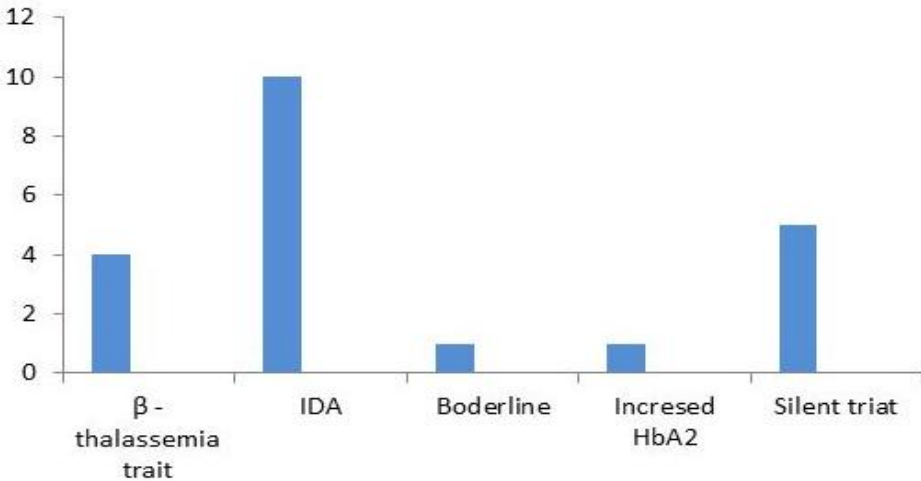


Fig. 5. Graph showing different results obtained after HPLC of 22 DMC students.

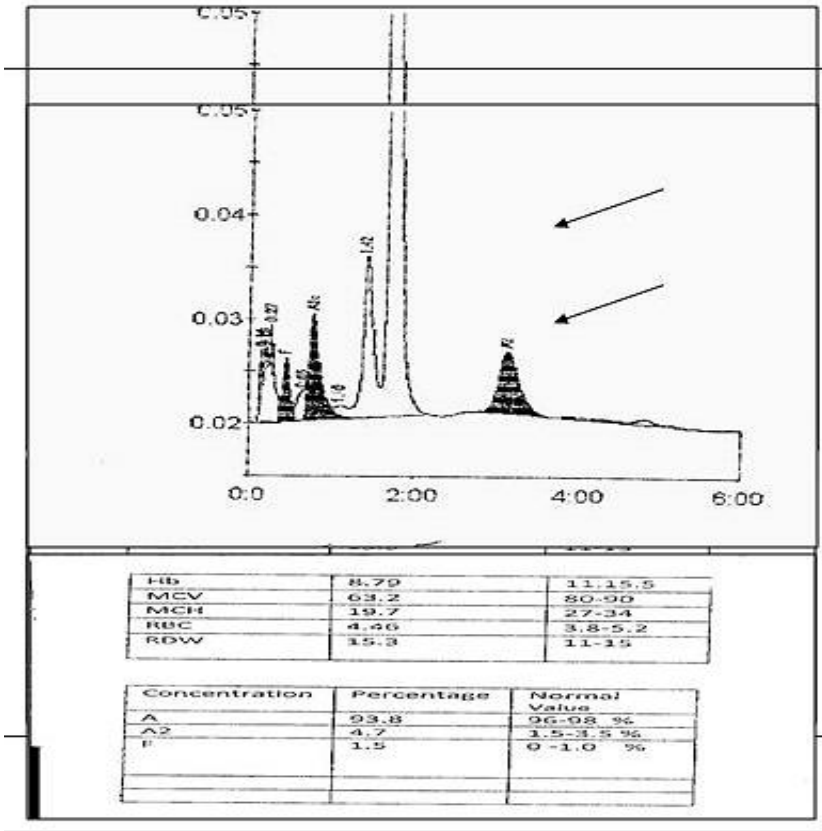


Fig. 6. hplc pattern of hemoglobin with beta thalassemia trait. note increased HbA2 (arrow)

4. CONCLUSIONS

In conclusion, NESTROFT, CBCs, and HPLC are sensitive, reliable, and real-time tests used to not only detect, but also confirm, the presence of β-thalassemia trait. NESTROFT can be used to initially screen the mass population, but due to the different sensitivities and handling errors reported, there is the chance an individual heterozygous for β-thalassemia trait will yield a false negative result. Therefore, it is essential to not rely solely on NESTROFT, but subsequently conduct CBCs to ensure no such individuals are overlooked. CBCs should be conducted especially before and after marriage. HPLC was used to confirm results. However, the results obtained demonstrate it is important to confirm positive HPLC results using DNA-based tests.

Because there is no documented registry available for β-thalassemia carriers in Pakistan, it is important to establish one and create awareness across the general population using media, newspapers, workshops, etc. A community-based preventive program could be initiated to identify β-thalassemia carriers. A countrywide premarital screening program would definitely aid in informing parents of β-thalassemia in offspring. Prenatal diagnosis and genetic counseling could



help those already facing this disorder. Although many programs to prevent thalassemia have been initiated, more screening is required at the government level to control the spread of this disorder and prevent cases of thalassemia from occurring in Pakistan.

For IDA, awareness programs should be initiated at all levels, including using the media, to inform people about healthy foods and proper iron intake. It has been suggested preventing and/or curing iron deficiency and IDA would improve cognitive, motor, and behavioral development among young affected children as IDA treatment improves concentration and attention span in women and children.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

None.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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