Original Article

Prevalence of Beta Thalassemia Trait in Semi-Urban Population of Karachi

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ABSTRACT

Objective: To evaluate the prevalence of Beta Thalassemia Trait in Semi-urban Population of Karachi.
Study Design: Comparative Cross-sectional study
Place and Duration of Study: This study was conducted at the Department of Medicine, Baqai Medical University, Karachi and its affiliated teaching hospitals in Karachi, from July 2021 to September 2021.
Materials and Methods: A total of 578 individuals were included in study, who were admitted in the medical ward and visiting out patients department (OPD) of the teaching hospital (Medical and obstetrics departments) of the Baqai medical university, Karachi. Patients with evidence of active blood loss, an acute or chronic inflammatory disorder, malignancy, recent surgery or pregnancy were excluded from the study. Complete blood count (CBC) was performed in all include individuals by using sysmex hematology analyzer. Mentzler's index was calculated and applied to identify the beta thalassemia carriers. Difference between RBC parameters of normal and carrier population was also compared.
Results: The 42 (7.2%) individuals among study population were suspected to have beta thalassemia trait on basis of Mentzler's index. In normal population Mentzler's index was 19.92±4.55 and in beta thalassemia trait (carrier) Mentzler's index was 10.15±3.33. It reliably detected microcytosis (positive predictive value of 95.2%). Statistically significant difference ($p<0.05$) of RBC parameters was noted between normal and suspected carrier groups.
Conclusion: RBCs parameters can reliably be used to screen and target thalassemia carriers.
Key Words: Thalassemia, Thalassemia screening, Thalassemia trait, Thalassemia carriers

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INTRODUCTION

Thalassemia, a group of diverse hemoglobin synthesis disorders, are regarded as the commonest genetic disorders. It is characterized by decreased or no globin chain synthesis. Traditionally it is classified as alpha or beta thalassemia, depending upon which synthesis is affected. More clinically meaningful terminology of transfusion dependent or non-transfusion dependent are becoming acceptable. Severity also depend upon presence of concomitant other mutations like sickle cell mutation or hemoglobin E. β- Thalassemia major (transfusion dependent thalassemia) disturbs both the patient and his or her β-thalassemia carrier parents.

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Apart from arranging regular blood transfusion, iron chelation therapy also costs a lot. Iron overload affects multiple organs. It is more common in Mediterranean region, Indian subcontinent and Middle East.[1.] Migration has changed epidemiology of Thalassemia to some extent, after addressing communicable diseases. [2] World Health Organization (WHO) has identified control of hemoglobin disorders, particularly β-thalassemia as priority. About 1.5% of the world's population is β- thalassemia carriers with 60,000 β-thalassemia births every year.[1] It is estimated 5 to 7% of Pakistan's population is β- thalassemia carriers.[3] A documentary registry need to be developed to get actual situation of β- thalassemia carriers.

At Pakistan treatment centers for thalassemia major patients are developed which provide regular blood transfusion and iron chelation therapy. Majority of these centers are developed by non-governmental organizations. Some of these centers are established in memory of β- thalassemia affectees by their families. Pakistan Bait-ul-Mall also support such patients. On the other hands measures to prevent incidence of β-thalassemia major appear less active.[4] Marriage between β- thalassemia carriers produce 25% probability of β- thalassemia major child. Family marriages, especially marriages among 1st degree cousins, are important contributory factor in this regard.

Pre-marriage screening of β- thalassemia carriers and counselling could be an important preventive step.[3] At Punjab province of Pakistan legislation is underway for β- thalassemia carrier screening before marriage. We lack thalassemia prevention strategy at national level. Punjab Thalassemia Prevention Program, a government funded initiative, is providing some service.[5] Even at school age, screening may be done and family may be informed about carrier status.

The different test or modalities like Naked eye single tube red cell osmotic fragility test (NESOFT), Cell Counter based formulas, hemoglobin electrophoresis and high performance liquid chromatography (HPLC) are used for β- thalassemia carrier screening with different degree of sensitivity and specificity.[6] The Naked eye single tube red cell osmotic fragility test (NESOFT) standardization is difficult as it may involve human visual error.[7] Cost is an issue with hemoglobin electrophoresis and high performance liquid chromatography (HPLC). They are also not routinely available. These two factors make hemoglobin electrophoresis and high performance liquid chromatography (HPLC) ineffective for mass screening. The screening of parameters by cell counter based and calculation of various indexes by mathematical formulas is fast, automated, cost effective and technically correct.[8] Use of such formula started back in 1973.[9] Currently, cell counters based formula are widely used in many parts of the world to screen β- thalassemia carriers. We also conducted a β- thalassemia carrier screening study in semi-urban population of Karachi, Sindh using cell counter based formulas. Other hematological parameters of Complete blood count (CBC) were also analyzed. This is an attempt to enroll and educate β-thalassemia carriers.

MATERIALS AND METHODS

This Comparative cross sectional study was conducted in the Department of Medicine, Baqai Medical University (BMU) in Karachi, from July 2021 to September 2021. A total of 578 individuals were included in study, who were admitted in the medical ward for evaluation of anemia and/or β- thalassemia carriers and visiting out patients department (OPD) of the teaching hospital (Medical and obstetrics departments) of the Baqai Medical University, Karachi. These patients were willing to participate in this study with given written consent too. Patients with history of active blood loss, an inflammatory disorder (acute or chronic), cancers or malignancy, history of recent surgery or blood transfusions were excluded from the study. This study was approved by the ethics committee of Baqai Medical University (BMU) /Ref: BMU-EC/01-2021, dated 12 February 2021. Sample size was calculated by Raosoft Sample size calculator. [10] For the study, three millimeter (ml) of venous sample (blood) was collected in Ethylenediamine tetraacetic acid (EDTA; anticoagulant) test tubes. The Complete blood count (CBC) including haemoglobin (Hb%), packed cell volume (PCV), red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was performed by using sysmex (XS-1000) hematology analyzer. The Red blood cell (RBC) or erythrocyte indices are useful for diagnosis of anemia or decreased hemoglobin concentration below normal (Hb-12-15.5 gm/dl in women and in males Hb-14-18 gm/dl) and types. Mentzler Index (1973): mean corpuscular volume (MCV)/ Red blood cell (RBC) count was applied to identify suspected Beta thalassemia carrier individuals. Mentzler Indexes (1973) quotient <13 was taken as cut off for Beta thalassemia carrier. If quotient less than 13 (Beta thalassemia carrier) and if the quotient of mentzers index more than 13 (iron deficiency anemia). This index is useful for diagnosis of type of anemia.[11] Statistical Package for the Social Sciences (SPSS) version 22 was used to analyze collected data. Relative descriptive statistics, frequency and percentages were calculated for categorical variables like gender and normal or suspected beta thalassemia carrier. Mean and standard deviation (Mean ± S.D) were calculated for quantitative variables like age, hemoglobin (Hb%), erythrocyte or RBC count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Independent sample t-test was applied to compare the difference in HB%, erythrocyte or RBC count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration between normal and/or suspected Beta thalassemia carrier individuals.

RESULTS

Out of 578 enrolled individuals, 300 patients (51.9%) females and 278 (48.1%) were male, female to male ratio was 1.08 (1.08:1). Among these 42 (7.2%) subjects were suspected to have beta thalassemia trait, on basis of Mentzer index. Mean and standard deviation were calculated in both normal and suspected beta thalassemia trait group for Hemoglobin (Hb%), Packed cell volume (PCV), red blood cell (RBC) or erythrocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Independent sample t-test showed statistically significant difference ($p< 0.05$) of Hemoglobin (Hb%) in normal individuals (11.12±2.53) and in beta thalassemia carrier (10.78±1.46), red blood cell (RBC) or erythrocyte count in normal individuals (4.12±0.71) and in beta thalassemia carrier (6.68±0.21), in mean corpuscular volume (MCV) in normal individuals (79.66±4.349) and in beta thalassemia carrier (55.57±3.01), in mean corpuscular hemoglobin (MCH) in normal individuals (26.27±4.197) and in beta thalassemia carrier (21.14±4.102), in mean corpuscular hemoglobin concentration (MCHC) in normal individuals (33.59±4.08) and in beta thalassemia carrier

(29.76±.85), and in Mentzler Index in normal individuals (19.92±4.55) and in beta thalassemia carrier (10.15±3.33). (Table. I). 40 out of 42 individuals of suspected beta thalassemia carrier had MCV≤ 76. (Positive predictive value = 95.24%).

Table No. I: Hematological parameters in normal and suspected beta thalassemia trait group

Parameter	Mean±SD in normal group (n=536)	Mean± SD In Suspected Beta Thalassemia Carrier (n=42)	p-value
Age (Years)	25.41±15.35	21.80±8.002	0.024
Hb (g/dl)	11.12±2.53	10.78±1.46	0.023
PCV(%)	34.38±18.63	37.19±9.87	0.05
RBC(10^6/µL)	4.12±0.71	6.68±0.21	<0.001
MCV(fl)	79.66±4.349	55.57±3.01	<0.001
MCH(pg)	26.27±4.197	21.14±4.102	<0.001
MCHC(g/dl)	33.59±4.08	29.76±.85	0.012
Mentzler Index	19.92±4.55	10.15±3.33	0.05

p-value< 0.05 significant, p-values>0.05 non-significant.

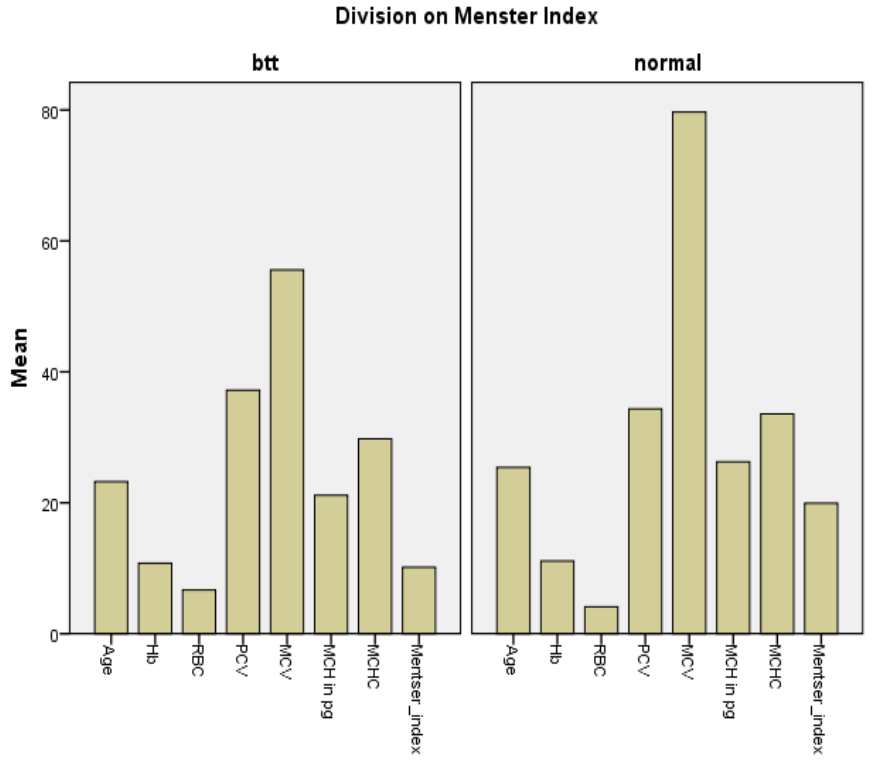

Figure No1: Graphical comparison of Hematological parameters in normal and suspected beta thalassemia trait group

DISCUSSION

Inexpensive diagnostic methods need to be developed for screening of β- thalassemia carriers, especially in developing or poor countries. People are reluctant for β-thalassemia carriers screening, not only due to financial reasons but also due to some social reasons. To declare someone suspected β- thalassemia carriers on basis of hemoglobin indices has long been discussed. Disproportionate decrease in mean corpuscular volume (MCV) and normal or slightly high red blood cell (RBC) count to raise suspicion of beta thalassemia carrier is well known.[5] One study published by Ferrara M et al in 2010 showed lower MCV value in β-thalassemia carriers than iron deficiency. When related to degree of anemia, β- thalassemia carriers had lower MCV than iron deficiency subjects with same hemoglobin level.[10,12] Parthasarathy V also concluded that mean corpuscular volume (MCV) < 76 fl and red blood cell (RBC) count > 4.9 × 10^{12}/L were associated with high probability of β- thalassemia carriers.[13] Our study had similar findings i.e MCV 55.57±3.01 and RBC count 6.68±0.21 in suspected β- thalassemia carriers. 95.24% of suspected β- thalassemia carriers had MCV <76, showing good positive predictive value. Karimi M et al concluded that MCH is more sensitive than MCV, for premarriage screening of β- thalassemia carriers(98.5 vs 97.6).[14] Study conducted by Bordbar E

et al also concluded that MCH is more sensitive than MCV.[15] In contrast to these results, Moafi A et al concluded MCV is more sensitive. They claimed MCV ≤ 80 can be used as cut off for screening β- thalassemia carriers. 4.1% of their study population, who were β-thalassemia carriers, had MCV ≥ 78 but ≤ 80. [16] Apart from β- thalassemia carriers and iron deficiency, other causes of microcytosis like alpha thalassemia trait, chronic diseases and sideroblastic anemia (although rare) may also alter sensitivity of these indices.[17] Therefore, instead of relying on single parameter, developing formula that include different indices can more sensitive to detect β- thalassemia carriers.[14] Different formula [(Mentzer index; MCV/RBC),(Srivastava;
MCH/RBC), (Ricerca; RDW/RBC) & (Green and King; MCV2×RDW/100 HB)] using RBC indices have varied sensitivities.[15] Pakistan is country with significant burden of thalassemia major. It can only be prevented or decrease by pre-marriage screening (Hb: electrophoresis) for beta thalassemia carrier. Accurate methods like hemoglobin electrophoresis and High Performance Liquid Chromatography (HPLC) are not suitable due to cost. Moreover, social norms do not permit or considers it bad, to think about disease on happy occasion. But use of Hb-electrophoresis or HPLC is not feasible due to cost and expertise needed. Apart from isolated hemoglobin indices analysis, to raise suspicion of β- thalassemia carriers, researchers have developed some formulas to raise suspicion about beta thalassemia carrier.

In our study, we utilized Mentzer index that showed raised suspicion of beta thalassemia carrier in 42 out of 578 individuals (7.2%) with positive predictive value of 95.24% for microcytosis. The results are comparable to previous studies. Importantly Pornprasert S et al found sensitivity and specificity of different varied among different populations.[18, 19] Variations at gene level may be responsible for that. Hence physician need to work to identify appropriate formula for their population. Algorithm may be developed to decide who could be candidate for hemoglobin electrophoresis on basis of CBC findings. This may ensure utilization of limited resources on right person. Kiss et al proposed an algorithm based on low MCV and ethnic background for screening purpose.[20] Their findings could not have been generalized because β- thalassemia carriers was more prevalent among one ethnic group of study population. But importantly they developed β-thalassemia carrier's probability table based on different values of MCV. They were in view that if β-thalassemia carrier's probability is > 20% based on their table, that person needs to be properly tested for β-thalassemia carriers. This may be the way to avoid over testing plus missing a person with β- thalassemia carriers. Amendolia et al probably used separation algorithms for first time, including support vector machine (SVM), to screen alpha and beta thalassemia carriers. It was in fact comparison of SVM and K-nearest neighbor (KNN) with a Multi layered Perceptron Classifier (MLP). Both techniques were 95% specific to distinguish thalassemia carriers from healthy individuals but MLP was slightly more sensitive than SVM.(95% vs 85%).[21] Roth et al also used single vector measurement for screening or raising suspicion for β- thalassemia carriers. They found all RBC parameter were significantly different between healthy individuals and β- thalassemia carriers. They developed a SVM formula using MCV and MCH, which was found highly sensitive. They compared their SVM results with different discrimination formula quoted in literature, and found that their SVM can reliably screen β- thalassemia carriers. They moved a step forward by comparing their formula with HPLC and Hemoglobin electrophoresis. They argued that both hemoglobin electrophoresis and HPLC can give false negative results if patient has iron deficiency or Hb level <9gm/dl. They found their formula was 99.56% sensitive in such cases.[22] Ahmed et al proposed algorithm for screening of β- thalassemia carriers in Pakistani population. They used one tube osmotic fragility test to raise suspicion of beta thalassemia carrier. Findings of one tube osmotic fragility test were compared to MCV and MCH. Test was found to be fairly sensitive. [23] Studies by Ansari et al[24] and Yazdani et al[25] showed similar results. This simple test, which does not need any expertise may be added to RBC parameters to make them more sensitive. It is recommended that Mass screening for beta thalassemia carrier at early age or during student life should be used and law for genetic counselling before marriage should be passed from parliament with strict implementation in our society that may help in prevention of thalassemia in Pakistan

CONCLUSION

RBCs indices can reliably raise suspicion of beta thalassemia carrier. Their reliability may further be enhanced by devising different formulas or their cut off for different populations due to genetic differences.

Author's Contribution:

Concept & Design of Study:	Adil Khan, Saqib ur Rehman
Drafting:	Amna Najeeb, Bushra Rabbani
Data Analysis:	Masooda Fatima, Saleem Ullah Abro
Revisiting Critically:	Adil Khan, Saqib ur Rehman
Final Approval of version:	Adil Khan, Saqib ur Rehman

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Diseases 2010;5(1):1-15.
2. Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of β-thalassemia. Eur J Haematol 2020;105(6): 692-703.
3. Asif N, Hassan K. Prevention of beta thalassemia in Pakistan. J Islam Med Dent Coll 2014;3(2):46-7.
4. Asif N, Hassan K. Management of thalassemia in Pakistan. J Islamabad Med Dental Coll 2016; 5(4):152-3.
5. Ghafoor M, Sabar MF, Sabir F. Prevention programs and prenatal diagnosis for beta Thalassemia in Pakistan: a narrative review. J Pak Med Assoc 2021;71(1):326-31.
6. Brancaleoni V, Di Pierro E, Motta I, Cappellini M. Laboratory diagnosis of thalassemia. Int J Laboratory Hematol 2016;38:32-40.
7. Sumera A, Ahmed S, Ali SA, Khanani R. Evaluation of NESTROFT as a marker of differentiation between?-Thalassemia Trait and Iron Deficiency Anemia. Int J Collaborative Res Internal Med Public Health 2012;4(8):0.
8. Risoluti R, Materazzi S, Sorrentino F, Bozzi C, Caprari P. Update on thalassemia diagnosis: new insights and methods. Talanta 2018;183:216-22.
9. Schriever H, Srivastava P. Differentiation of thalassaemia minor from iron deficiency. The Lancet 1973;302(7821):154-5.
10. Raosoft. Raosoft Sample Size Calculator. Raosoft, Inc., Seattle 2004.http://www.raosoft. com/ samplesize.html.
11. Abro S, Soomro MS, Saleem Q, Sultana S, Jafri I, Jafri F. Gender based Comparison of Body Mass Index, Red blood Cell Indices and Mentezer index in Medical Students. Med Forum 2019;30(3): 61-65.
12. Jameel T, Baig M, Ahmed I, Hussain MB, bin Doghaim Alkhamaly M. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pak J Med Sci 2017;33(3): 665.
13. Parthasarathy V. A search for beta thalassemia trait in India. Turkish J Haematol 2012;29(4):427.
14. Karimi M, Rasekhi A. Efficiency of premarital screening of beta-thalassemia trait using MCH rather than MCV in the population of Fars Province, Iran. Haematologia 2002;32(2):129-33.
15. Bordbar E, Taghipour M, Zucconi BE. Reliability of different RBC indices and formulas in discriminating between β-thalassemia minor and other microcytic hypochromic cases. Mediterranean J Hematol Infectious Diseases 2015;7 (1).
16. Moafi A, Valian S, Nikyar Z, Zeinalian M, Momenzadeh M, Rahgozar S. Prevalence of minor β-thalassemia based on RBC indices among final suspected individuals in premarital screening program referred to genetic laboratories. Int J Hematol-Oncol and Stem Cell Research 2010:23-7.
17. Sankar VH, Arya V, Tewari D, Gupta UR, Pradhan M, Agarwal S. Genotyping of alpha-thalassemia in microcytic hypochromic anemia patients from North India. J Applied Genetics 2006;47(4):391-5.
18. Rashwan N, El-Abd Ahmed A, Hassan M, Mohammed M, Helmi Bakri A. Hematological indices in differentiation between iron deficiency anemia and beta-thalassemia trait. Int J Pediatr 2022;10(1):15285-15295.
19. Pornprasert S, Panya A, Punyamung M, Yanola J, Kongpan C. Red cell indices and formulas used in differentiation of β-thalassemia trait from iron deficiency in Thai school children. Hemoglobin 2014;38(4):258-61.
20. Kiss TL, Ali MA, Levine M, Lafferty JD. An algorithm to aid in the investigation of thalassemia trait in multicultural populations. Archives Pathol Laboratory Med 2000;124(9):1320-3.
21. Amendolia SR, Cossu G, Ganadu M, Golosio B, Masala GL, Mura GM. A comparative study of k-nearest neighbour, support vector machine and multi-layer perceptron for thalassemia screening. Chemometrics and Intelligent Laboratory Systems 2003;69(1-2):13-20.
22. Roth IL, Lachover B, Koren G, Levin C, Zalman L, Koren A. Detection of β-thalassemia carriers by red cell parameters obtained from automatic counters using mathematical formulas. Mediterranean J Hematol Infectious Diseases 2018; 10(1).
23. Ahmed S, Saleem M, Modell B, Petrou M. Screening extended families for genetic hemoglobin disorders in Pakistan. New Engl J Med 2002;347(15):1162-8.
24. Ansari SH, Hanifa A, Saleem A, Ali SM, Hussain Z, Zohaib M, et al. Sensitivity and specificity of Single-Tube Osmotic Fragility Test and its different methods as screening test for thalassemia trait: an alternative to expensive laboratory tests for resource-limited countries. Eur J Haematol 2014;93(6):516-20.
25. Yazdani MS, Ahmed S, Bhatti FA, Azim W. One tube osmotic fragility test: a screening test for microcytic red cells. PAFMJ 2008;58(1):45-50.