ORIGINAL ARTICLE

DIAGNOSTIC ACCURACY OF RDWI TO DIFFERENTIATE IRON DEFICIENCY ANEMIA AND THALASSEMIA TRAIT IN BORDERLINE HBA2

MALEEHA ALI KHAN[1], MARYAM SALMAN[2], NAGHMANA MAZHER[3], FAREEHA IMRAN[4], SHABNUM BASHIR[5]

[1]Senior Demonstrator, [2] Post-graduate Resident, [3] Associate Professor, [4] Senior Demonstrator, [5] Professor

[1-3,5]Fatima Jinnah Medical University, Lahore, [4]Postgraduate Medical Institute, Lahore

ABSTRACT

Background: Thalassemia is an inherited hemoglobin disorder which is characterized by the reduction or absence of α or β chains due to mutations in these genes. Reduced hemoglobin synthesis in IDA (iron deficiency anemia) results in microcytic hypochromic red blood cells. Microcytic hypochromic anemia is hallmark of beta thalassemia trait and the patient is usually asymptomatic. It is important to differentiate it from other causes of iron deficiency anemia (IDA) which also shows similar peripheral blood picture. The final diagnosis is based on quantification of HbA2 and serum ferritin levels. If the level of HbA2 is more than 3.5%, then diagnosis of beta thalassemia trait is made. The problem arises with the borderline HbA2 i.e, 3.1% - 3.4%. There is also diagnostic difficulty when concomitant iron deficiency anemia is present.

Objectives: To determine the diagnostic accuracy of RDWI in borderline HbA2 to distinguish the beta thalassemia trait from IDA and to diagnose beta thalassemia trait when genetic analysis is not easily available.

Methods: It was a cross sectional validation study. A total of 90 patients were included in this study, having HbA2 between 3.1-3.4%. Tests for serum ferritin, serum iron and total iron binding capacity (TIBC) were done on all the samples. RBC morphology, RBC count, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and RDW of all samples were noted. RDWI of all samples was calculated. Mutational analysis by ARMS (Amplification Refractory Mutation System) PCR was done for confirmation of β thalassemia trait.

Results: Out of 90 samples with borderline HbA2, 30 (33%) cases were diagnosed as β thalassemia trait. Out of these, 15(50 %) had concomitant IDA along with beta thalassemia trait and 15 (50%) were non-iron deficient beta thalassemia carriers. 43 samples were not iron deficient and were also negative for β thalassemia trait, whereas 17 samples had only IDA without beta thalassemia trait. The sensitivity of RDWI was 76.6% and specificity was 56.6% in borderline HbA2.

Conclusion: RDWI is a good indicator to differentiate between IDA and β thalassemia trait (sensitivity 76.6%, specificity 56.6%).

Key words: Iron deficiency anemia, Red cell, Mean cell volume, Mean cell hemoglobin, Borderline A2.

How to cite this article: Khan MA, Salman M, Mazher N, Imran F, Bashir S, Diagnostic Accuracy of RDWI To Differentiate Iron Deficiency Anemia and Thalassemia Trait in Borderline Hba2. Pak Postgrad Med J 2023;34(2):77-82

DOI: https://doi.org/10.51642/ppmj.v34i01.548

Correspondence to: *Naghmana Mazhar*
Associate Professor, Fatima Jinnah Medical University, Lahore, Pakistan.

Email: *dnm4cf@gmail.com*

INTRODUCTION

The most prevalent genetic condition with an autosomal recessive pattern of inheritance is thalassemia. Adult hemoglobin's beta globin chains are not synthesized at all or are synthesized with diminished intensity in beta thalassemia. The beta thalassemia gene is present in about 4% of the world's population. The incidence is higher in the Mediterranean region, Middle East, Southeast Asia, and Indian subcontinent, and 79% of those affected are from Asian nations[1]. Globally, 1.5% of people are thalassemia carriers[2]. Between 1% and 7% of Pakistanis have the beta thalassemia trait. Given that both present with microcytic

hypochromic anemia, it is challenging to distinguish between IDA and thalassemia trait.[3]

The most frequent nutritional deficiency in both industrialized and developing countries is reported to be iron deficiency anemia, or IDA. IDA affects 15 to 20 percent of people worldwide[4]. IDA is the most prevalent kind of anemia in Pakistan as well[5]. Reduced hemoglobin synthesis in IDA results in microcytic hypochromic red blood cells. It is brought on by decreasing iron intake, decreased or increased need for iron, and persistent gastrointestinal bleeding or menorrhagia. Both beta thalassemia trait and IDA have a microcytic hypochromic blood appearance, and it can be challenging to distinguish between the two conditions. To make an appropriate diagnosis, a number of different hematological and biochemical tests must be performed[6]. It is crucial to distinguish between the two in order to correctly diagnose a person's beta thalassemia carrier status and avoid the birth of a child with severe thalassemia by providing appropriate genetic counselling. The assessment of iron metabolism, including serum ferritin, TIBC, and iron levels, is used to make the diagnosis of IDA. When ß thalassemia trait is present, a high RBC count and hypochromic, microcytic anemia are both present.[7] RDW calculates the RBCs' size variance. As it is above the reference range in iron deficiency anemia, it can help distinguish IDA from the thalassemia trait. Hb A2 quantitative measurement (normal range 2.4-3.2%) is the most helpful test. For such situations, estimation of Hb A2 can be employed as a diagnostic tool.[8] It ranges from 3.6 to 7% in thalassemia trait. Borderline HbA2 values between 3.2 and 3.4% require more investigation.[9] The gold standard for thalassemia diagnosis is DNA analysis. The beta globin genes exhibit a variety of mutations and deletions. The first and most crucial laboratory test for determining beta thalassemia trait is a complete blood count. In nations like Pakistan, where both beta thalassemia trait and iron deficiency are prominent, red cell characteristics are examined as part of a baseline inquiry. On the basis of hemoglobin electrophoresis alone, a microcytic hypochromic anemia in a patient with a family history of thalassemia shouldn't be deemed normal. By estimating the iron profile, the possibility of concurrent IDA and beta thalassemia trait should be ruled out. In these situations, PCR should be performed.[9] However, expensive testing like Hb analysis and mutation analysis are needed to confirm the diagnosis. To distinguish the thalassemia trait from IDA in individuals with microcytic hypochromic anemia, a variety of discriminant formulas have been created. Red blood cell distribution width index (RDWI), Mentzer Index, Srivastava Index, Green and King Index, Shine and Lal Index, and red cell count are some of these indices. [11]

OBJECTIVES

To determine the diagnostic accuracy of RDWI in borderline HbA2 to distinguish the beta thalassemia trait from IDA and to diagnose beta thalassemia trait when genetic analysis is not easily available.

METHODS

It was a cross sectional validation study and conducted in the Main Laboratory of Punjab Thalassemia Prevention Program (PTPP) at Sir Ganga Ram Hospital (SGRH) Lahore. A total of 90 patients from Sir Ganga Ram Hospital were included in this study.

Inclusion Criteria:

- 2years of age.
- HbA2 between 3.1%- 3.4%.

Exclusion Criteria:

- History of transfusion in previous 4 months.
- Patients on iron therapy.
- Any variant detected on HPLC (eg HbE, HbD, HbS.)

Ninety subjects fulfilling the inclusion criteria were enrolled. After consent from the patient/guardian, personal and background information was recorded on the prescribed performa. A complete family history of patient with special emphasis on the presence of beta thalassemia in other family

Three ml of venous blood was taken in an EDTA vial. This sample was used within six hours for Hb, RBC count, and hematocrit, MCV, MCH, MCHC, RDW and RDWI. Additional 3 ml of blood was taken in another EDTA vial and was used for HPLC. 3 ml of blood was taken in vacutainer with no anticoagulant. This sample was used for estimation of serum iron, TIBC and serum ferritin.

Complete Blood Count (CBC): EDTA blood sample was run in Automated Hematology Analyzer (Sysmex KX-21).

Serum Iron: Serum iron was measured by Modified calorimetric method with Lipid Clearing Factor (LCF) also called CAB Method.

Reference value of Serum Iron in males is 75-175 µ gm/dl and in females is 28-162µ gm/dl (Burtis, C.A and Bruns, D.E.,2015)

Total Iron Binding Capacity (TIBC): Reference values range of TIBC is 250-410µgm/dl (49-69µmol/l) (Burtis, C.A and Bruns, D.E.,2015)

Serum Ferritin: Quantitative measurement of serum ferritin is based on monoclonal antibodies directed against ferritin molecules and are detected by ELISA.

Reference value of serum ferritin in men is 15-300 µg/l while in women is 15-200µg/l (Bain, B.J. et.al.,2017)

Quantification of the hemoglobin fractions by High-performance liquid chromatography (HPLC): Quantification of the hemoglobin fractions was done by High-performance liquid chromatography (HPLC) on the samples with Biorad Variant using beta thalassemia short program.

Molecular Analysis: Blood sample was collected in vacutainers containing ethylene diamine tetra acetic acid (EDTA). DNA was extracted from peripheral leucocytes by using blood mini kit (Qiagen, GmbH, Hilden, Germany).

DNA was amplified using the polymerase chain reaction (PCR): primer sequence used for most common mutation in Pakistan is given below in Table 1.

The data was entered and analyzed by using IBM SPSS version 20.0 (Statistical Package for Social Sciences). The quantitative variables were given in the form of Mean ± Standard Deviation (SD). The sensitivity, specificity and diagnostic accuracy of RDWI, was determined by using two by two table.

RESULTS

A total of 90 patients with borderline HbA2 (3.1-3.4) were included in the study.

The mean age of total cohort was 21 ±12.6 with range of 2-55 years ,47 (052.2 %) were males and 43 (47.8 %) were females.

Out of these 40 were found to be having concomitant IDA and 50 cases showed no evidence of iron deficiency. On the basis of iron studies and PCR a total cohort were divided into four subgroups.

- IDA without beta thalassemia trait (n=25)
- IDA with beta thalassemia trait (n=15)
- Beta thalassemia trait without IDA (n=15)
- Normal No IDA No beta thalassemia trait (n=35)

Table: 1 Primers designing for primary mutations by ARMS (Amplification Refractory Mutation System) PCR

No	Primer	Sequence
1	Control-F	5'-CAATGTATCATGCCTCTTTGCACC
2	Control-R	5'-GAGTCAAGGCTGAGAGATGCAGGA
3	Common-1	5’-ACCTCACCCTGTGGAGCCA
4	Common-2	5’-CCCCTTCCTATGACATGAACTTAA
5	Fr 8-9 (+G) M	5’-CCTTGCCCCACAGGGCAGTAACGGCACACC
6	IVSI-5 (G-C) M	5’-CTCCTTAAACCTGTCTTGTAACCTTGTTAG
7	Fr 41-42 (-TTCT) M	5’-GAGTGGACAGATCCCCAAAGGACTCAACCT
8	Cd 15 (G-A) M	5’-TGAGGAGAAGTCTGCCGTTACTGCCCAGTG
9	Cd 5 (-CT) M	5’-ACAGGGCAGTAACGGCAGACTTCTCCGCAG
10	IVSI-1 (G-T) M	5’-GATGAAGTTGGTGGTGAGGCCCTGGGTAGG
11	Cd 30 (G-C) M	5’-TAAACCTGTCTTGTAACCTTGATACCTACT
12	Cd 30 (G-A) M	5’-TAAACCTGTCTTGTAACCTTGATACCTACC
13	Fr 16 (-C) M	5’-TCACCACCAACTTCATCCACGTTCACGTTG
14	Cap+1 (A-C) M	5’-ATAACAGCATCAGGAGTGGACAGATAGATC
15	Del 619bp-F	5'-GAGTCAAGGCTGAGAGATGCAGGA
16	Del 619bp-R	5’-TGAGGAGAAGTCTGCCGTTACTGCCCAGTA
17	Common-1	5’-ACCTCACCCTGTGGAGCCA
18	Common-2	5’-CCCCTTCCTATGACATGAACTTAA

Table: 2 Hematological Parameters in total cohort

Parameter	Number	Minimum	Maximum	Mean	SD
Hb (g/dL)	90	4.3	16.3	11.8	2.11
RBC (m/mm^2)	90	2.8	7.1	5.2	87427
MCV (fL)	90	55.6	96	75.46	8.8
MCH (pg)	90	14.3	31.4	22.7	3.22
HbA2 (%)	90	3.1	3.4	3.2	0.11
RDW (%)	90	11.0	25.0	14.83	2.5

Hematological parameters showed no significant difference between beta thalassemia patients having iron deficiency and without iron deficiency in study group. RDWI between these two groups also showed no significant difference as shown in Table 3.

COMPARASON OF BIOCHEMICAL PARAMETERS AMONG THALASSEMIA TRAIT GROUP WITH AND WITHOUT IDA: In confirmed cases of beta thalassemia trait with and without iron deficiency anemia the comparison of biochemical tests is shown in Table 4:

MUTATION ANALYSIS OF STUDIED POPULATION: The DNA was extracted from all 90 samples having borderline HbA2 i.e., 3.1 -3.4. Out of these 90 thirty were confirmed as beta thalassemia heterozygotes on PCR. The most common mutation seen among the study group was IVS (1-5) 13(43%) followed by CAP+1 9(30%), Fr 8-9 5(117%), cd5 2(7%). The

least common mutation was Cd30 with just 1(3%) in given group as shown in Figure: 1

SENSITIVITY AND SPECIFICITY OF RDWI: The sensitivity, specificity and diagnostic accuracy of RDWI, was determined by using two by two table (Table 4).
Sensitivity of RDWI in total cohort with borderline HbA2 was 76.6%. It means three-fourth individuals will be diagnosed as having beta thalassemia trait and one fourth will be missed. Specificity was 56.6% i.e only half of the individuals were correctly identified for not having thalassemia trait. The positive predictive value is the percentage of people who actually have the trait and RDWI positive predictive value was 47% and the negative predictive value was 82.9%. The diagnostic accuracy of RDWI is 63.3%

Table: 3 RDWI and Hematological Parameters in different study groups

		No.	Min	Max	Mean	S. D	Std. Error Mean	*p* value
HB (g/dL)	IDA	15	7.1	15.5	10.8933	2.23781	.57780	0.116
	NIDA	15	7.1	15.6	11.7857	2.00187	.53502	
RBC (m/mm^2)	IDA	15	3.4	7.1	5.7027	1.05588	.27263	0.941
	NIDA	15	4.4	7.1	5.7273	.73977	.19101	
MCV (fL)	IDA	15	55.6	86.8	66.0600	9.27283	2.39424	0.393
	NIDA	15	57.7	84	68.9800	9.18035	2.37036	
MCH (pg)	IDA	15	14.3	27.7	20.1200	4.23981	1.09471	0.65
	NIDA	15	16.0	23.9	20.7333	2.97745	.76878	
RDW (%)	IDA	15	13.0	22.3	17.4267	2.71068	.69990	0.09
	NIDA	15	13.0	18.8	15.9467	1.95224	.50407	
RDWI	IDA	15	149.2	318.7	205.6067	44.54018	11.50023	0.353
	NIDA	15	149.2	249.9	192.6533	29.17175	7.53211	
HBA2 (%)	IDA	15	3.1	3.4	3.2333	.11751	.03034	0.357
	NIDA	15	3.1	3.4	3.2733	.11629	.03003	

IDA-Iron deficiency anemia NIDA –Non-Iron deficiency anemia *p* value < 0.05 is considered statistically significant

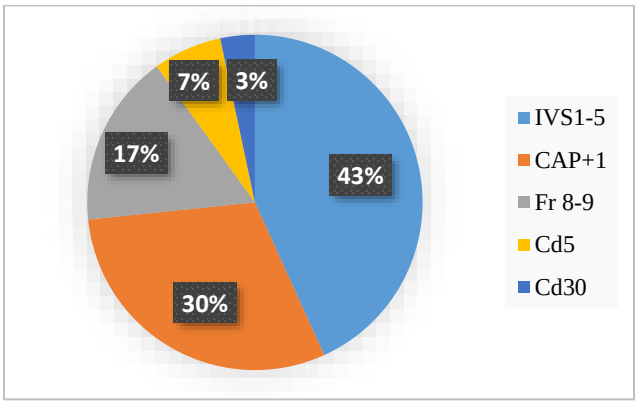

Figure: 1 Relative frequency of β thalassemia Mutations

Table: 4 Diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value of RDWI

Parameter	% Age
Diagnostic accuracy	63.3%
Sensitivity	76.6%
Specificity	56.6%
PPV	46.9%
NPV	82.9%

DISCUSSION

Beta thalassemia trait is extremely common in Pakistan.[12] In several research, the -thalassemia characteristic is distinguished from IDA using particular haematological measures and combinations of absolute values.[13] In the majority of beta thalassemia patients, HbA2 exceeds 3.5%. On occasion, a borderline HbA2 reading between 3.2 and 3.4% is discovered. In high incidence regions like Turkey, Italy, Greece, Saudi Arabia, the United Arab Emirates, India, and Pakistan, borderline HbA2 is fairly prevalent.[14]
These cases could be caused by IDA or alpha thalassemia together with thalassemia trait. Reduced HbA2 levels can be identified in the presence of alpha thalassemia mutations, according to a study by Al Jaffer et al. This results from the binding of beta chains with alpha chains rather than delta chains. In the HbH illness, this is shown. In sideroblastic anaemia, severe IDA, chronic illness anaemia, acquired HbH condition, and lead poisoning, HbA2 is also decreased. As a result, certain cases of beta thalassemia can be undiagnosed because HbA2 levels do not rise to the required level. Therefore, before a conclusive diagnosis of a beta thalassemia carrier is made, HbA2 levels must be associated with other criteria. In a similar manner, a different study discovered that HbA2 is decreased in IDA and increased in beta-TT and alpha-thalassemia trait. However, a HbA2 of less than 3.5% is a poor predictor of beta-TT. Delays in the nuclear maturation of red blood cell progenitors, such as those that occur in

megaloblastic anemia, can also have an impact on HbA2 levels. and by consuming substances that impair nuclear maturation. These patients have increased HbA2 levels but do not have beta thalassemia. [15]

According to a Colaco study from 2021, borderline HbA2 is widespread and one-third of those people have a molecular abnormality. 20% of borderline A2 individuals, according to the authors, carried beta-thalassemia. Because routine screening may miss the diagnosis in people with borderline HbA2, which could lead to the birth of a child with thalassemia major, this condition should be thoroughly assessed. [16]

The goal of our study was to evaluate the diagnostic performance of RDWI in borderline HbA2 to discriminate between iron deficiency anaemia and the beta thalassemia phenotype. Such trials in patients with borderline HbA2 levels are quite rare. Our research was done in collaboration with PTPP, which provides free thalassemia diagnosis testing as well as extended family carrier screening. Prenatal diagnostics and voluntary premarital screening are also provided.

A total of 90 patients with borderline HbA2 were used in this investigation. 47 (52.2%) of them were men and 43 (47.8%) were women. There was no bias based on gender. This gender distribution was comparable to one from Pakistan, where 54% of the population was male and 45% female. [17]

In accordance with the data gathered, PCR was used to determine that 33% (30/90) of the participants had borderline HbA2 and a thalassemia characteristic. A different study's conclusion that 35% of those with borderline HbA2 cases had a molecular abnormality is consistent with this one. Similar findings were found in different research of the Kelantan population, where 31% of borderline HbA2 samples revealed beta thalassemia gene alterations. According to a study from Pakistan, it can be challenging to diagnose patients of beta thalassemia that have borderline HbA2.[18]

In our investigation, there was no statistically significant difference between beta-thalassemia features with and without concurrent IDA. Comparable research from India found no appreciable differences in MCV, MCH, MCHC, and RDW between beta-thalassemia carriers who had and did not have an iron deficit. [19]

According to a study from India, beta-TT dramatically reduced MCV and MCH compared to IDA. The number of red blood cells also rose in individuals with the beta thalassemia trait.[20] According to a study from Israel, patients with beta thalassemia trait and concurrent iron deficiency anemia did not have RDW levels that were statistically significant. Duzenli observed comparable outcomes, concluding that RDW is not very helpful in differentiating between IDA and beta thalassemia trait.[21]

The sensitivity and specificity of RDWI were, respectively, 76.6% and 56.6% in our investigation. This indicates that 75% of those with borderline HbA2 and the beta thalassemia trait had their diagnoses made accurately by RDWI. RDWI had a 56.6% specificity, which means that 34 out of 60 were indeed negative. According to the 46.9% positive predictive value, 46.9% of people who tested positive actually had the condition. Since 82.9% of those who tested negative did not have the condition, the negative predictive value was 82.9%. In our investigation, the RDWI's diagnostic Accuracy was 63.3%. It means that when thalassemia trait is present, it can be detected in borderline HbA2 levels up to roughly 60%.

The sensitivity and specificity in a study on RDWI of the Saudi population in patients with HbA2 more than 3.5% were 94% and 88%, respectively. Similar findings were found in an Indian study, which demonstrated that the RDWI was the best marker to identify the beta thalassemia trait from IDA, with 97.5% of patients receiving the correct diagnosis.[22] In our investigation, RDWI in borderline cases was not a very good predictor of the trait beta-thalassemia compared to the studies described above. Other reasons of borderline HbA2 may be to blame, which we have not ruled out. According to one study, borderline HbA2 is the outcome of beta thalassemia and alpha thalassemia.[23]

In our study, molecular analysis revealed 43% IVS1-5 mutations, with CAP +1 mutations coming in second place (30%), followed by Fr 8-9 mutations (16%), Cd 5 mutations (6%), and Cd 30 mutations (3%). The same findings were seen in an Indian study that showed a borderline HbA2 to have a 44% IVS 1-5 and 30% CAP+1 defect.[24] In our investigation, borderline HbA2 was found even in people with normal iron metabolism who had mutations like IVS (1-5) and Fr 8-9. The cost of treating beta-thalassemia major is a significant public health burden in a developing nation like Pakistan. The cost of adequate blood transfusions with sufficient iron chelation is high. The majority of people cannot afford a bone marrow transplant. Treatment of the disease is far inferior than prevention. The risk of having a kid with thalassemia can be significantly reduced via proper screening and preventive programs. Preventative steps include genetic counselling, premarital screening, and prenatal diagnosis, to name a few. The general population should be informed about and inspired to participate in volunteer screening. Prenatal diagnosis should be explained to couples who are at risk.[25] Therefore, it is advised that in nations with a high prevalence of thalassemia disease, every effort should be made to make a proper diagnosis, especially in high-risk families, and that the diagnosis should, whenever possible, be verified by molecular analysis.

CONCLUSION

RDWI, though a good indicator to differentiate between iron deficiency anemia and beta thalassemia trait (sensitivity 76.6% specificity 56.6%) But in all cases of borderline HbA2 genetic analysis should be done especially if a partner is tested positive for beta thalassemia trait to confirm the diagnosis.

REFERENCES

1 Ehsan H, Wahab A, Anwer F, Iftikhar R, Yousaf MN. Prevalence of transfusion transmissible infections in beta-thalassemia major patients in Pakistan: a systematic review. Cureus. Cureus. 2020; 12(8): e10070.

2 Saxena R, Chamoli S, Batra M. Clinical Evaluation of Different Types of Anemia. World. 2018 Jan; 2(1):26-30.

3 Shukla S, Singh D, Dewan K, Sharma S, Trivedi SS. Antenatal carrier screening for thalassemia and related hemoglobinopathies: A hospital-based study. J Med Soc 2018; 32:118-22.

4 De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, Daar S, Wali Y, Yassin M, Soliman N, Sobti P. β-thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. Mediterr J Hematol Infect Dis. 2017; 9(1): e2017018.

5 Norton P, Araújo N, Pinho P, Gomes JC, Silva C, Gama C, Barbosa M, Ferreira P, Cunha S, Moreira S, Martins S. Diagnosis, treatment, and work impact of iron deficiency anemia in a Portuguese urban community. Porto Biomed J. 2020; 5(4): e064.

6 Habib N, Abbasi SU, Aziz W. An analysis of societal determinant of anemia among adolescent girls in Azad Jammu and Kashmir, Pakistan. Anemia. 2020; 21:9.

7 El-Shanshory MR, Sherief LM, Hassab HM, Ragab SM, Yahia S, Mansour AK, Ahmed AS, Abdou SH, Helmy AM, Watany MM, Gad ALllah AM. Prevalence of iron deficiency anemia and beta thalassemia carriers among relatives of beta thalassemia patients in Nile Delta region, Egypt: a multicenter study. J Egypt Public Health Assoc 2021; 96:1-8.

8 Suhaimi SA, Zulkipli IN, Ghani H, Abdul-Hamid MR. Applications of next generation sequencing in the screening and diagnosis of thalassemia: A mini-review. Front Pediatr. 2022; 10: e1015769.

9 Zhou Y, Zhang J, Wang C, Zhou L, Zhou L, Ou D, Peng D. Application of HbA2 levels and red cell indices-based new model in the differentiation of thalassemia traits from iron deficiency in hypochromic microcytic anemia Cases. Int J Lab Hematol. 2020; 42(5):526-32.

10 Lee YK, Kim HJ, Lee K, Park SH, Song SH, Seong MW, Kim M, Han JY. Recent progress in laboratory diagnosis of thalassemia and hemoglobinopathy: a study by the Korean Red Blood Cell Disorder Working Party of the Korean Society of Hematology. Blood Res. 2019; 54(1):17-22.

11 Ali G, Tariq MA, Shahid K, Ahmad FJ, Akram J. Advances in genome editing: the technology of choice for precise and efficient β-thalassemia treatment. Gene Ther. 2021; 28(1-2):6-15.

12 Maskoen AM, Reniarti L, Sahiratmadja E, Sisca J, Effendi SH. Shine & Lal index as a predictor for early detection of β-thalassemia carriers in a limited resource area in Bandung, Indonesia. BMC Med Gene. 2019; 20(1):1-6.

13 Zakaria NA, Islam MA, Abdullah WZ, Bahar R, Mohamed Yusoff AA, Abdul Wahab R, Shamsuddin S, Johan MF. Epigenetic insights and potential modifiers as therapeutic targets in β–Thalassemia. Biomolecules. 2021; 11(5):755.

14 Eren R, Karışmaz A, Aslan C, Doğu MH, Altındal Ş, Yokuş O, Suyanı E. Beta thalassemia minor: patients are not tired but depressed and anxious. Medical Principles and Practice. 2021; 30(1):69-72.

15 Colaco S, Nadkarni A. Borderline HbA2 levels: Dilemma in diagnosis of beta-thalassemia carriers. Rev Mut Res. 2021; 788:e108387.

16 Yousafzai YM, Wahid QU, Khan S, Mir A, Khan A, Raziq F. Co-existing iron deficiency/overload in beta-thalassemia trait. J Pak Med Assoc. 2019; 69(6):806-10.

17 Ghafoor M, Sabar MF, Sabir F. Prevention programmes and prenatal diagnosis for beta thalassemia in Pakistan: A narrative review. J Pak Med Assoc. 2021; 71(1):326-31.

18 Arora S, Rana D, Kolte S, Dawson L, Dhawan I. Validation of new indices for differentiation between iron deficiency anemia and beta thalessemia trait, a study in pregnant females. Int J Sci Rep. 2018; 4(2):26.

19 Jahangiri M, Rahim F, Malehi AS. Diagnostic performance of hematological discrimination indices to discriminate between βeta thalassemia trait and iron deficiency anemia and using cluster analysis: Introducing two new indices tested in Iranian population. Sci Rep. 2019; 9(1):e18610.

20 Düzenli Kar Y, Özdemir ZC, Emir B, Bör Ö. Erythrocyte indices as differential diagnostic biomarkers of iron deficiency anemia and thalassemia. J Ped Hematol/Oncol. 2020; 42(3):208-13.

21 Wahan A, Huchannavar TK, Pnbsp SH. Reliability of Red Cell Indices for Differential Diagnosis of Beta Thalassemia Trait and Iron Deficiency Anemia. RGUHS J Allied Health Sci. 2021; 1(2). 10-17.

22 Satthakarn S, Panyasai S, Pornprasert S. Molecular characterization of β-and α-globin gene mutations in individuals with borderline hb a2 levels. Hemoglobin. 2020; 44(5):349-53.

23 Nadkarni AH, Gorakshakar AC, Sawant PM, Italia KY, Upadhye DS, Gorivale MS, Mehta PR, Hariharan P, Ghosh K, Colah RB. The phenotypic and molecular diversity of hemoglobinopathies in India: a review of 15 years at a referral center. International J Lab Hematol. 2019; 41(2):218-26.

24 Saleem, N., Anwar, A., Shahid, N.U.A., Saleem, R., Saleem, Z., et al. Perception of Parents of Thalassemic Child to Thalassemia in Pakistan. Cureus. 2021; 13(8): e17615.