International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.9, pp 909-917, 2017

Comparison study on Band 3 Mutation and Fragility of Erythrocytes in β Minor Thalassemia

Hidayat[1]*, Bidasari Lubis[2], Yahwardiah Siregar[3]

[1]Department of Biochemistry, Faculty of Medicine, University of Sumatera Utara, Indonesia.
[2]Department of Paediatry, Faculty of Medicine, University of Sumatera Utara, Indonesia.
[3]Biomedic Magister Programme, Faculty of Medicine, University of Sumatera Utara, Indonesia.

Abstract : Thalassemia is a hereditary hemolytic anemia which is caused by globin gene disorder (mutation). Thalassemia is widely distributed in Mediterranean and around the equator area. In Indonesia, thalassemia is the most common cause of hemolytic anemia intracorpuscular. Stability of erythrocytes is influenced by state and function of cytoskeleton proteins. It is also influenced by band 3 protein. Band 3 protein mutation lead the damage of erythrocytes while circulation in narrow capillaries. Therefore, thalassemia with band 3 mutation lead the early erythrocytes destruction and anemia. This study was carried out to find β minor thalassemia and to compare of band 3 mutation and erythrocytes fragility on thalassemia and non thalassemia β minor. This was an analytic descriptive study with cross-sectional approach. Population of this study were students of University of Sumatera Utara with Mentzer Index value < 13. Band 3 protein mutation was detected by PCR .
Prevalence rate of β minor thalassemia was 32% and β minor thalassemia with band3 mutation was 2%. Band 3 mutation was found in one of 16 subjects with and β minor thalassemia with erythrocytes fragility was increased. It needs specific and sensitive method for screening and diagnosed of and β minor thalassemia.
Keywords : Band 3 protein, fragility test, Mentzer Index, thalassemia β minor.

Introduction

Thalassemia is a hereditary hemolytic anemia disease inheritedrecessively from both parents to their child due to disorder (mutation) of globin gene. Thalassemia is classified intoα-thalassemia due to deficiency of α-globin chain synthesis and β-thalassemia due to β-globin chain deficiency synthesis.[1,2] Thalassemia was distributed in Mediterranean and around the equator area.In Indonesia, thalassemia is the common cause of intracorpuscular hemolytic anemia. World Health Organization (WHO) 2006, reported 7% of the world's population are thalassemia carriers, and a total of 300,000 - 500,000 babies were born with thalassemia each year. A total of 1.67% people in the world suffering from thalassemia. The prevalence of thalassemia is the highest in tropical countries, but due to the high migration, the disease can be found around the world.[2]

Globin protein forms hemoglobin. Globin is a protein around heme and binds with it to protect heme molecule. Hemoglobin is a molecule with 4 polypeptide chains, 2 α-globin chains and 2 β-globin chains. The differences between these four globin chains are in the total and structure of the amino acids. α-globin chain

consists of 141 amino acids whereas β-globin chain consists of 146 amino acids. These 4 globin chains bind heme group that contains Fe atom. Heme group bound to the 4 globin chains form hemoglobin molecule.[2]Thalassemia characterized by the lack or decrease of one or more of hemoglobin chains.[3]

Mutations in globin gene lead to globin chain synthesis disorder. It can be reduced or no synthesis of globin chains. Total of globin chains become imbalanced, leads some unmatch globin chains which will precipitate out, attach to erythrocytes membrane and lead membrane auto-oxidation. Auto-oxidation lead to change of cell membrane erythrocytes structure. The cell membrane erythrocytes become more rigid lead to reduced of deformability of cell membrane erythrocyte.[5,6,7]

The stability of the erythrocytes is strongly influenced by state and function of cytoskeleton proteins, which basically consists of actin, spectrin, ribbons 4.1, 4.2, and ankyrin. Cytoskeleton proteins are crucial to the ability of erythrocytes to flow through capillaries that are smaller than its diameter to prevent damages. In addition, the stability of erythrocytes was also influenced by the band 3 protein, an integral transmembrane erythrocytes protein. Cytoplasmic domain (hydrophilic) band 3 protein interacts with spectrin which is mediated by ankyrin. Band 3 protein is also important as an anion exchanger protein, i.e. HCO_3^- and Cl^- ions through the erythrocytes membrane. Since, the role of protein band 3 as an anion exchange, erythrocytes perform CO2 transport function.[8,9,10]

Band 3 protein mutation may affect its function, result disruption of homeostasis of erythrocytes then affect to ability of erythrocytes to defend itself to prevent damage when circulating on narrow capillaries. Thus, mutations in band 3 erythrocyte membrane protein also associated with increased premature destruction of erythrocytes and anemia.[11]

The abnormality of band 3 protein has been found in some blood disorders such as ovalocytosis, acanthocytosis and spherocytosis. In previous study, it has also been identified that patient of βminorthalassemia also has band 3 protein mutation. Existences of rigidity accompanied by reduced of deformability of erythrocyte membranes of thalassemia that is similar to ovalocytosis, leads to thought that damage of thalassemia proteins of erythrocyte membrane is also caused by mutation in protein-coding of band 3 genes. If this happens will worsen the damage of erythrocyte membrane that is caused by load of oxidation.[12]

Characteristics of thalassemia patients who were hospitalized at RSUP Haji Adam Malik (HAM) Medan in 2008 were patients aged 6-15 years old are 65.8%, 63.3% male, 83.3% pale complaints, 88.3% types of β thalassemia and 60% requiring transfusion. Until now, no specific study to examine possibility of thalassemia patients who are treated and handled in the RSUP HAM Medan also have mutation in protein-coding genes band 3. In addition, many cases of mild anemia which is treated in RSUP HAM Medan as well as at other health services in Medan have not been investigated whether the anemia cases are caused of thalassemia, mainly minor thalassemia.

There is only a few data of patients with thalassemia who are diagnosed, since β minor thalassemia patients with mild clinical symptoms of anemia rarely visit hospital or other health services for treatment. Number of patients with β thalassemia is higher than α thalassemia . The objectives of this study were to find out patients of β minor thalassemia of 1800 new students of University of Sumatera Utara (USU) and to compare band 3 mutation and erythrocyte fragility on students of USU with β minor thalassemia and non with β minor thalassemia.

Currently, screening individuals suspected of suffering from Thalassemia determined by using the Mentzer Index (MI) value. MI was first discovered in 1973 by Mentzer. MI is the results of Mean Corpuscular Volume (MCV) value divided by Red Blood Cell Count (RBC) value. MI value < 13 was determined as suspect of β minor thalassemia and MI value > 13 is determined as suspect patients with Iron Deficiency Anemia. This study also applied MI value as a screening tool for determined suspected subject of thalassemia.[14] Band 3 mutation was detected from DNA analysis by Polymerase Chain Reaction (PCR) method.

Materials and method

This was an analytic descriptive study. Population of this study were students of USU from academic year of 2013/2014 and 2014/2015 and data was obtained from USU Health Centre's medical records of regular

blood test during new student's orientation period. This study were counducted on December2013until November 2014. Mentzer Index (MI) is obtained from results of complete blood examination: Mean Corpuscular Volume (MCV) divided by the value of Red Blood Cell Count (RBC). Study population were subject with inclusion criterias, those who hass been suspected as β minor thalassemia by measurement of MCV < 80 fL, MCH < 26 pg, Mean Corpuscular Hemoglobin Concentration (MCHC) < 32 g/dL and MI < 13. Samples are subject with inclusion criteria of MI vaue< 13 and willing to be involved in the study. Of 1800 subjets , 66 subjects were found with MI value < 13. Only 50 subjects wanted to participate in this studyand signed the informed consent.

Gradually, the subject's blood samples which was obtained then putin 3 tubes.Two tubes were sent to ProdiaLaboratory for examination of hemoglobin type by using HPLC method and 1 tube was used for fragility test and DNA isolation. The study subjects with HbA2 > 3.5% were diagnosed as patients with β minor thalassemia or trait β thalassemia. Fragility test was conducted as soon as possible before 24 hours since blood samples were taken to avoid the test result was not accurate.

DNA blood samples were also isolated directly on the same day or the next day after being stored at 4°C. After isolating DNA from blood samples, the purity of isolated DNA was determined by measuring the concentration using a spectrophotometer at a wavelength of 280 and 260 nm. DNA from the samples that had been isolated and purified, proceed to the next stage of multiply DNA fragment that encoded the forming of erythrocytes of band 3 protein by using PCR. On preeliminary examination, PCR was performed on a DNA sample by adjusting various annealing temperature, ranging from 62°C, 63°C, 64°C, 65°C, 66°C, and 67°C. The purpose was to get the optimal annealing temperature in PCR process, and from this preliminary investigation, 62°C was determined as the optimal temperature for annealing process. This study used the same primer whichis used to identify abnormality in ovalositosis.

In data processing, subjects were divided into two groups. Group I consisted of subjectswho had MI value < 13 and diagnosed as β minor thalassemia based on Hb HPLCexamination, while group II consisted of subjectswho had MI value < 13 but were not diagnosed as β minor thalassemia based on the results of Hb HPLC. Non-probability sampling was used with simple random sampling technique. The samples were collected until the size samples was enough. All study subjects should be explained and asked for informed consent.

Results and Discussion

Table 1 shown that total of study subjects were 50 students, consist of 25 males (50%)and 25 females (50%). Characteristics of study subjects based on sex in group I, consisted of 8 males (16%) and 8 females (16%). In group II consisted of 17 males(34%) and 17 females (34%).

Table 1. Characteristics of Study Subjects by Sex

Group	Male		Female		Total	
	n	%	n	%	N	%
Group I	8	16	8	16	16	32
Group II	17	34	17	34	34	68
Total	25	50	25	50	50	100

***Group I :β minor thalassemia. *Group II: non β minor thalassemia**

Table 2 shown that all study subjects with total of 50 subjects, consisted of ethnic of Chinese, Javanese, Mandailing, Batak, Malay, Aceh, Minang, Ambon, Karo, Nias, and Sunda. In Group I, consisted of six ethnics i.e. sequentially from the largest number were Javanese, Malay, and Chinese, follow by ethnics of Minang, Aceh, and Batak.

Table 2. Characteristics of Study Subjects by Ethnic

Ethnic	Group I		Group II		Total	
	n	%	n	%	N	%
Aceh	1	6,25	2	5,88	3	6
Ambon	-	-	1	2,94	1	2
Batak	1	6,25	5	14,71	6	12
Javanese	4	25	6	17,65	10	20
Karo	-	-	1	2,94	1	2
Mandailing	-	-	8	23,53	8	16
Malay	4	25	1	2,94	5	10
Minang	2	12,5	1	2,94	3	6
Nias	-	-	1	2,94	1	2
Sunda	-	-	1	2,94	1	2
Tionghoa	4	25	7	20,59	11	22
Total	16	100	34	100	50	100

***Group I :β minor thalassemia. *Group II: non β minor thalassemia**

To see if there was a difference between group I and group II, mean difference test was performed from several components of routine blood test that consisted of Hemoglobin (Hb), RBC, Hematocrit, MCV, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), RDW Index, MI value and NaCl concentration on total hemolysis.

Tabel 3 shown, the values ofHb, MCH, MCHC and NaCl concentration on total hemolysis, the mean difference test used Mann-Whitney test, whereas for other components used independent t-test. Mean difference test of components of routine blood test, MI and NaCl concentration on total hemolysis, the probability value > 0.05. It meant that there was no difference in components of routine blood tests, MI and value of concentration of NaCl when haemolysis between group I and group II.

Table 3.Mean Difference Test Results of each Component of Routine Blood Tests: Mentzer Index Value and NaCl Concentration on total haemolysis.

Blood Component	Group I (n=16)		Group II (n=34)		Probability
	X	SD	X	SD	
Hb	13,24	2,41	14,29	4,04	0,553
RBC	5,94	0,71	6,03	0,78	0,685
Hematokrit	40,64	6,52	43,14	10,34	0,380
MCV	68,11	4,66	70,73	9,32	0,191
MCH	22,24	2,73	23,32	4,10	0,435
MCHC	32,58	3,02	32,89	2,39	0,827
RDW	14,53	1,41	14,81	2,15	0,644
RDW Index	167,81	20,90	173,77	27,49	0,447
Mentzer Index	11,58	1,19	11,78	1,12	0,512
NaClConcent.	0,34	0,04	0,36	0,04	0,307

***Group I :β minor thalassemia. *Group II: non β minor thalassemia**

Electrophoresis results of PCR gene AE1 for all study subjects was shown in Figure 1 and 2. Of 50 study subjects, only 1 subject (2%) had gene mutation of band 3 protein and this subject was member of group

I. There was no one in group II who had gene mutation of band 3 protein. If only according to group I, percentage of band 3 protein gene mutation was 6.25%.

Figure 1. Result of electroforesis gel agarose 2% 32 well : DNA marker (lane 1), negative control (lane 2), DNA sample 1-14 (lane 3-16 line 1), DNA sample 15-28 (lane 3-16 line 2).

Figure 2. Result of electroforesis gel agarose 2% 32 well : DNA marker (lane 1), positive control (lane 2), negative control (lane 3), DNA sample 29-41 (lane 4-16 line 1), DNA sample 42-50 (lane 4-12 line 2), DNA positive sample (lane 8 line 1)

Figure 3 showed, fragility test of group I, had various NaCl concentration from 0.32% to 0.42%. Fragility test of study subjects who hadband 3 protein mutations showedvery low concentration value, reached concentration of NaCl at 0.28%oncomplete haemolysis.

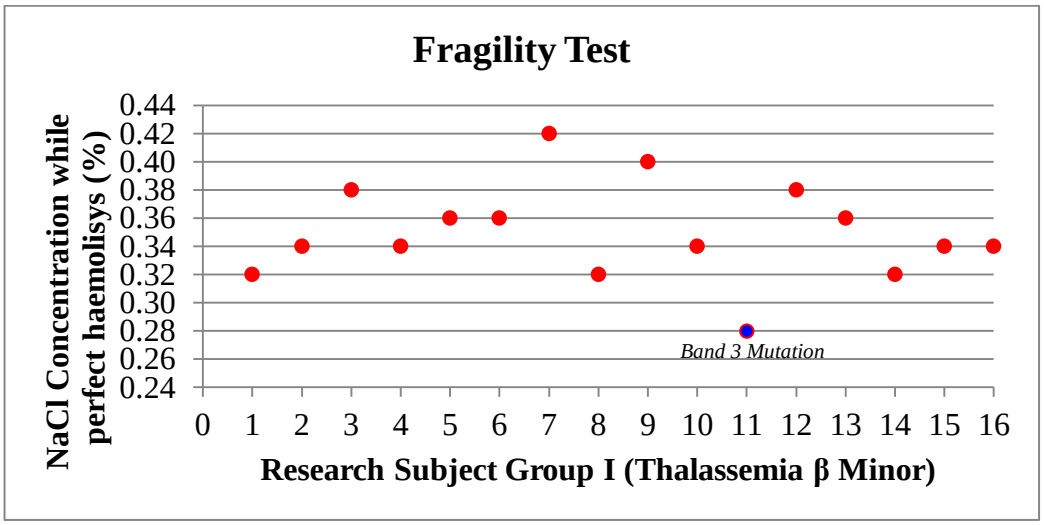

Figure 3. Fragility Test Result Chart of Research Subject Group I (β minor Thalassemia)

Figure 4 showed, fragility test of group II, had a various concentration value of NaCl from 0.32% to 0.42% on a complete haemolysis.

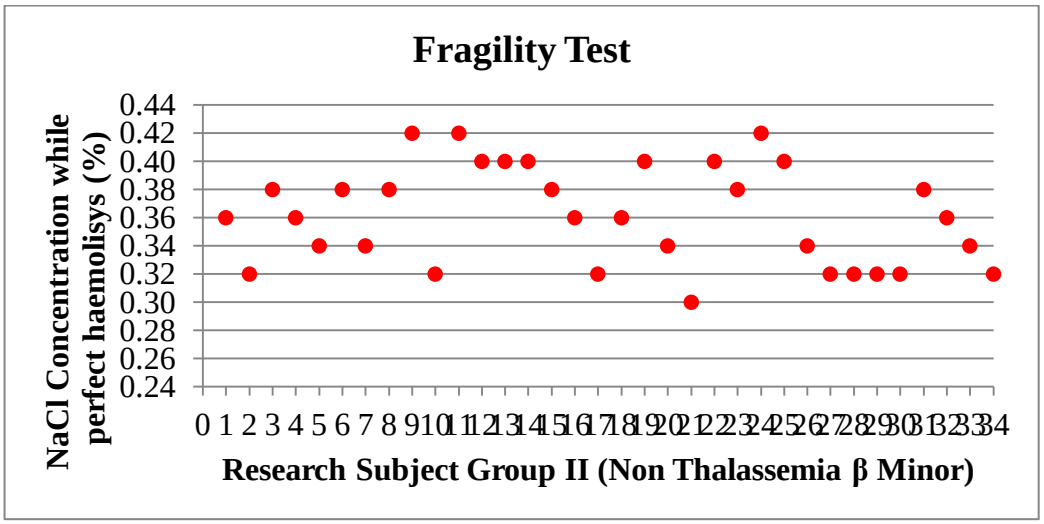

Figure 4. Fragility Test Result Chart of Research Subject Group II (non β minor Thalassemia)

In this study, number of students whohad MI value < 13 were 66 (3.67%) of 1800 students. The previous study which concentrate on MI valueof a population had different value. The study population was taken from medical records of Microbiology Department of the University Hospital AHEPA in Thessaloniki, Greece, it obtained 225 people who had MI value < 13 of 493 samples (45.64%).[13] The difference percentage because population of that study were already had microcytic anemia, whereas in this study the initial population was healthy subject without considering whether they were suffering from anemia or not.

Of 66 subjects who had MI value < 13, total of 50 subjects were examined for their type of hemoglobin by using HPLC method. Study subjek with HbA2 value > 3,5% diagnosed as β minor thalassemia and was classified in group I. A total of 16 subjects (32%) were β minor thalassemia. Comparing to previous studies, percentage of β minor thalassemia in population with MI value < 13 was still quite small. The previous study conducted by Ntaois at Department of Microbiology at AHEPA University Hospital Thessaloniki Greece, found out 223 patients with β minor thalassemia of 225 subjects withMI value < 13.[13] Beside this, these studies also obtained about 150 subjects suffering from β minor thalassemia with MI value > 13. This indicated that MI value was not very sensitive to determine β minor thalassemia. The other study suggested that from few factors for screening β minor thalassemia patient, MIvalue has not the highest sensitivity, but RBC and RDW Index

does.[14]However, in thisstudy, RBC or RDW Index value did not show a significant difference between subject withβ minor thalassemia and subject non β minor thalassemia.

There were 8 males and 8 females β minor thalassemia subjects in this study. Many literatures do not mention that there is a correlation between prevalence of βminor thalassemia and sexes. This is consistent to the theory stated that β thalassemia is notx-linked inherited, but it is known that structure and synthesis of Hb is regulated by a gene cluster that located on chromosome 11.[7]

In this study, each value of Hb, RBC, hematocrit, MCV, MCH, MCHC, RDW, and RDW Index in group of β minor thalassemiawere not too different from previous similar studies.[13]It suggested that Hb, MCV, MCH, MCHC, RDW, and RDW Index value could not be used to distinguish between β minor thalassemia patients and non β minor thalassemia.

Some literatures revealed that the clinical manifestations of patients with β thalassemia minor / anemia trait had mild or no anemia and had MCV value 60 to normal.The results of this study were consistent with this statement. The mean score ofHbvalue of β minor thalassemia was 13.24 which suggested that β thalassemia minor patient did not have an anemia. Therefore, we could not determine whether a person suffers from β minor thalassemia only refer to Hb value. The mean value of MCV in β minor thalassemia was 68.11. It was below of the normal MCV value. However, according to the difference test, although the MCV value is low, it could not be determined as β minor thalassemia patient.[15]

In this study erythrocytes fragility could not be seen in group of β minor thalassemia. Difference test of erythrocytes fragility test showed no significant difference between β minor thalassemia and non β minor thalassemia group. Thus, the fragility test suggested also did not give a satisfied result to distinguish between β minor thalassemia and non β minor thalassemia.

To find gene mutation of protein band 3, which also caused ovalositosis, this study used a primer that produces PCR products like gene protein band 3 that located in exon 11 from the 1098th to 1272ndbase, the length of gene obtained is 175 base pairs (bp). In ovalositosis patients, results of electrophoresis PCR product which used that primershowed two bands with size on 175 bp and 148 bp. The emergence of the second band (bottom) sized 148 bp indicates the deletion of 27 bp (Figure 1).[16]

The deletion occured in exon 11, 1197thbase to 1225thbase.The 27 bp deletion cause 9 amino acids that build band 3 protein on ovalositosis disappeared, it make balance impairment of erythrocytes cytoskeleton protein structure and movement disorder. This is caused by mobility of band 3 protein was decrease and band 3 protein-cytoskeleton protein conjunction was increase then lead the rigidity of the membrane increased.[17]

As in ovalositosis, band 3 protein gene mutation that found in patients with β-thalassemia might cause disruption of movement of erythrocyte cell cytoskeleton proteins, so that cell membrane became rigid. If a patient with β thalassemia also has band 3 protein mutation, consequently the condition of erythrocytes would become more severe. It is because the cell membrane was rigid because of the oxidation inthalassemia condition. The decreasing deformability of erythrocyte membranes on thalassemiawhich due to the rigidity of membrane would cause the erythrocytes damaged while flowing through narrow capillaries. This factor causes erythrocytes age of thalassemia patient shortenthen resulted anemia.

This study used the same primer that used to detected abnormality in ovalositosis. It is in order to know whether the membrane rigidity state and ability of erythrocyte membrane deformability in patients with β minor thalassemia is due to band 3 protein gene mutations like in ovalositosis. Of 50 samples, electroforeses of DNA PCR product showed that there was 1 sample had two bands. The first band was located above 150 bp and the second band was below 150 bp when compared to DNA marker.This was also appeared on DNA from sample of ovalositosis patient.[16] The DNA PCR product of ovalositosis sample was used as positive control in this study.Refer to the rule above, only 1 subject of β minor thalassemia who had band 3 mutation. It meant, the comparison of subject with β minor thalassemia who had band 3 mutation and all subjects who had β minor thalassemia was 1:16. The comparison of subject who had β minor thalassemia with band 3 mutation and all subjects either β minor thalassemia and β minor thalassemia was 1:50.

Fragility test of study subjects who had band 3 protein mutations showed very low NaClconcentration, at 0.28% on complete haemolysis. This value was lower than mean fragility test value of others who also in

group β minor thalassemia (0.34% NaCl on total hemolysis). This ssuggested that erythrocytes of this subject was very rigid. Fragility test of group non β minor thalassemia showed various value of NaCl, from 0.32% to 0.42% on complete haemolysis. These wasa normal fragility test value.There is no subject hada very low NaCl concentration. This suggested that erythrocytes of group β minor thalassemia did not have an increase in rigidity.

The study which was conducted in Medical Faculty University of Indonesia on 2004, obtained 6 subjects who had band 3 protein gene mutation of 50 subjects suffering from βthalassemia major (12%). The difference percentage because the study subjects were taken from β major thalassemia patients. In this study, only one subject (6,25%) who hadband 3 gene mutation and this subject came from β minor thalassemia group. Therefore,it was possibile that low NaCl concentration on total haemolysis is caused by band 3 protein gene mutation and in addition because the β minor thalassemia itself.[18]

Conclusion

This study investigated that the prevalence rate of suspects of β minor thalassemia was 3,67%. The prevalence rate of β minor thalassemia found as 32%. The blood components Hb, RBC, hematocrit, MCV, MCH, MCHC, RDW and RDW Index value between β minor thalassemia patients and non β thalassemia minor did not show any significant differences even they had the same MI value < 13. Thus, suggested that the determination of β minor thalassemia patient, could not be refer to those components value. Fragility test did not give a satisfied method to distinguish between β minor thalassemia and non β minor thalassemia since in this study it did not show any significant differences. The only 1 subject with band 3 mutation had a very rigid membrane. The prevalence rate of β minor thalassemia with band 3 mutation was 2%. The comparison of subject β minor thalassemia who had band 3 mutation with all subjects of β minor thalassemia was 1:16. The comparison of subject of β minor thalassemia with band 3 mutation with all subjects was 1:50. Only 1 subject had band 3 mutation so the comparison is 1:50. It needs a strategy and sensitive technique for screening of β thalassemia minorsince thalassemia patients with mild clinical symptoms of anemia rarely visithospital or other health services for treatment.The diagnosis must be determined by DNA analysis (molecular diagnosis), a diagnosis that can directly show the defects in patient's DNA structure like band 3 mutation.

References:

1. Maskoen AM, Sofiana D, Reniarti L. Optimization of Pyrosequencing method to detect IVS1-NT5 β-Globin gen mutation in β-thalassemia., International Journal of PharmTech Research. 2017; 10(2) : 96-102.
2. Abdoerrachman. Bukuilmukesehatananak 1. Cetakan ke-11.PercetakanInfomedika. Jakarta; 1985.
3. Al-Luhaybi AI. The detection of antibody titer in thalassemia patients in Al-Najaf Iraq.International Journal of PharmTech Research. 2017; 10(1) : 465-469.
4. Higgs DR, Bowden DK. Clinical and laboratory features of the alfa-thalassemia syndromes. In: Steinberg MH, Forget BG, Higgs DR, Nagel RL, editors. Disorders of hemoglobin: Genetics, pathophysiology and clinical management. Cambridge UK: Cambridge University Press; 2004. p. 431-69.
5. Suryohudoyo P. KapitaSelekta: Ilmukedokteranmolekular. 2000.
6. Shinar E, Shalev O, Rachmilewitz EA. and Schrier SL. Erythrocyte membrane skeleton abnormalities in severe β thalassemia. Blood. 1987; 70: 158-4.
7. Olivieri NF. The β-thalassemias. The New England Journal of Medicine. 1999. 341(2): 99-109.
8. Voet D, Voet JG. 1995. Biochemistry. Ed2. John Wiley & Sons, Inc., New York.
9. Hamasaki N. The role of band 3 protein in oxygen delivery by red blood cells. Indian Journal of Clinical Biochemistry. 1999;14(1): 49-58.
10. Wang DN. Band 3 protein : structure, flexibility and function. FEBS Letters 1994; 346: 26-31.
11. Dhermy D, Bournier O, Bourgeois M and GrandchampB. The red blood cell band 3 variant associated with dominant hereditary spherocytosis causes defective membrane targeting of the molecule and a dominant negative effect. Molecular Membrane Biology. 1999; 16: 305-312.
12. Cappellini MD, Tavazzi D, Duca L, Graziadei G, Mannu F, Turrini F, *et al*. Metabolic indicators of oxidative stress correlate with hemichrome attachment to membrane, band 3 aggregation and erythrophagocytosis in β- thalassemia intermedia. British Journal of Haematology. 1999; 104: 504-12.

13. Ntaois G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G, *et al*. Discrimination indices as screening tests for β-thalassemic trait. Ann Hematol. 2007; 86: 487-491.
14. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Padiatr Int. 2002; 44(6): 612-616.
15. Rund D, Rachmilewitz E. β thalassemia. The New England Journal of Medicine. 2005; 353: 1135-1146.
16. Jarolim P, Palek J, Amato D, Hassan K, Sapak P, Nurse GT. Deletion in erythrocyte band 3 gene in malaria-resistant southeast asianovalositosis. ProcNatiAcadSci 1991; 88: 11022-26.
17. Mohandas N, Winardi R, Knowles D, Leung A, Parra M, George E. Molecular basis for membrane rigidity of hereditary ovalocytosis, a novel mechanism involving the cytoplasmic domain of band 3. J Clin Invest. 1992; 89: 686-692.
18. Dewajanthi AM. Identifikasia danyakelainan gen protein pita 3 padamembranseldarahmerahpenderita thalassemia β di pusat thalassemia RSCM/ FKUI. 2004.

*****