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Diagnosis of thalassemia using fluorescence spectroscopy, auto-analyzer, and hemoglobin electrophoresis — A prospective study

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ABSTRACT

Background: Hemoglobinopathies (HgP) are prevalent in certain regions of the world. The World Health Organization estimated that 5% of the world's population is a carrier of the potentially pathological hemoglobin (Hb) gene.
Methods: This study aimed to compare the performance of fluorescence spectroscopy, a simple and inexpensive method, with that of conventional techniques for diagnosing thalassemia. The red blood cell (RBC) counts and levels of Hb, HbA, HbA_2, and HbS were estimated via conventional methods of complete blood count and Hb electrophoresis to diagnose thalassemia.
Results: The RBCs and Hb, particularly the average values of HbA and HbA_2, were lower in patients with thalassemia than in the normal controls. These hematologic parameters were also analyzed via fluorescence spectroscopybased on fluorescent biomolecules including tyrosine (275 nm), tryptophan (290 nm), nicotinamide adenine dinucleotide (NADH) (370 nm), flavin adenine dinucleotide (FAD) (450 nm), and porphyrin (585–635 nm). In thalassemia patients, all these parameters were above the normal range, primarily due to abnormal depression of NADH and elevation of FAD.
Conclusion: Thalassemia canbe diagnosed via a fluorescent spectral method with an accuracy of 85% for blinded groups. This method may be useful for screening patients and reducing the cost of diagnosis in many rural countries.
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Introduction

Hemoglobin (Hb) is an essential component of red blood cells (RBCs) that binds with oxygen in the lungs and plays an important role in the circulation of oxygenated blood throughout the body [1]. One RBC contains approximately 270 million Hb molecules [2]. Blood disorders such as thalassemia are generally characterized by the imbalanced synthesis of globin chains. Approximately 5% of the world's population is a carrier of Hb disorders [3]. One of the main reasons for this higher incidence in some populations is consanguineous marriages within blood relations [4]. The Hb (HbA) molecule in a normal adult has four subunits, i.e., two alpha and two beta ($\alpha 2\beta 2$). Patients with thalassemia do not produce sufficient HbA because of mutations in the genes that code for the globin proteins, thereby disrupting the generation of normal alpha or beta polypeptide chains [5]. Thalassemia occurs when one or more of these subunits are faulty or deleted [6]. Alpha thalassemia is the result of aberrations in one or more alpha globin genes leading to a loss of alpha chains [7]. Likewise, beta thalassemia is caused by mutations in one or more beta globin genes and the resultant loss of beta chains [8,9]. Alpha and beta thalassemia disease (TD) can occur in major, minor, and intermediate genetic forms and can also interact with the abnormal Hb proteins present in the same individuals [10].

Abbreviations: HbA, Hemoglobin A; HbA2, Hemoglobin A2; HbS, Hemoglobin S; HbE, Hemoglobin electrophoresis; TD, Thalassemia disease; TT, Thalassemia trait.

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Alpha thalassemia is more prevalent in the tropical and subtropical regions where malaria is endemic, and the migration of people from these regions has made this condition a common clinical problem in North America, North Europe, and Australia [11]. Meanwhile, beta thalassemia is more common in the Mediterranean, African, Middle-East, Central Asia, and Indian subcontinent regions [12].

Although many methods are currently used to diagnose thalassemia, the clinical history and hematologic evaluation via a complete blood count (CBC) using an automated analyzer provides a better read out of the various blood components for both sexes in all age groups. In addition, RBC morphology and reticulocyte counts help differentiate patients with thalassemia from those with other hematologic conditions [13]. Electrophoresis is the most common method for Hb evaluation. Different hemoglobin such as HbA, HbA_2, and HbS have different charges, which make them move at different speeds in the gel via an electric field [14]. Aberrations in the Hb proteins alter their charges, which can be identified by changes in their speed of migration in the gel. Recently, cation exchange high-performance liquid chromatography has become a popular method for the detection of many Hb subtypes [15]. Further, polymerase chain reaction has been used for the detection of gene deletions in thalassemia [16].

Spectral diagnosis using fluorescence spectroscopy which is based on the interaction of photons with fluorescent blood biomolecules is a newly evolving technique for the detection, diagnosis, and monitoring of diseases such as different types of cancers [17,18], sickle cell anemia, and thalassemia [19]. The present study was performed to evaluate and compare the performance of fluorescence spectroscopy with those of standard modalities, i.e., CBC using automated hematology analyzer and electrophoresis, in the diagnosis of thalassemia.

Materials

We analyzed 50 blood samples each from normal controls and patients (median age of both samples are 32)with thalassemia from the King Khalid Medical City, Riyadh, Saudi Arabia. Blood samples were collected after obtaining written informed consent. The research protocol was approved by the Institutional Review Board (Approval No.: E-17-2267).

Patients

Conventional methods such as CBC and Hb electrophoresis were used to confirm the diagnosis of thalassemia. After diagnosis, blood samples of 50 patients with thalassemia (15 with TT and 35 with TD; 30 men, 20 women) were collected and analyzed using the spectral technique to measure the levels of specific fluorescent biomolecules.

Controls

Fifty healthy volunteers including 30 men and 20 women with no history of TD, TT, sickle cell diseases, sickle cell trait, iron deficiency, diabetes mellitus, alcoholism, or any other blood-related illness were selected as normal controls.

Methods

Auto-analyzer

A total of 5 ml of blood was collected in EDTA coated tubes via venipuncture in both patients and healthy controls. RBC distribution width and other CBC parameters such as Hb, hematocrit, platelets, lymphocytes, and mean corpuscular volume were measured via an automated blood analyzer (Sysmex Automated Hematology KX-21 N, Sysmex Corporation, Kobe, Japan).

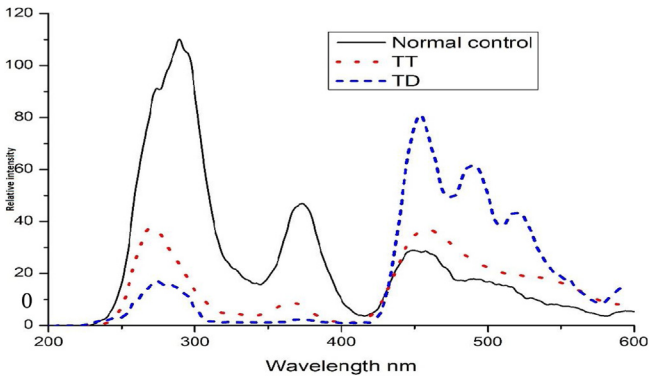


Fig. 1. Synchronous excitation spectra of plasma of control, TT and TD samples.

Hemoglobin electrophoresis

The Kohn Model U77 (Shandon Southern Instruments Ltd., Surrey, UK) standard horizontal tank attached on 78 mm × 150 mm cellulose acetate membranes (Shandon Celagram strip) with a Vokam Power Pack (Shandon Southern Instruments, Ltd., Surrey, UK) was used for Hb electrophoresis.

Hemolysates were prepared by adding one drop of whole blood to 0.5 ml of working hemolyzing reagent, mixing it and incubating for 10 min at room temperature. The concentration of hemolysates (approximately 0.25 g/dl) was matched visually, and more blood was taken for samples with low Hb concentrations [20].

Spectrofluorometric analysis

A spectrofluorometric analysis was performed on the blood plasma samples and the RBCs of patients with TT and TD as well as healthy controls. The techniques and instruments used have been described previously [17–21].

Statistical analysis

The data were presented as a mean ± standard deviation. Comparisons between TT and TD were performed using one-way analysis of variance. Statistical hypotheses were tested with the significance of $p < 0.05$.

Results

The synchronous excitation spectrum (SXS) of the controls and patients with TT and TD is shown in Fig. 1a–c. The five main peaks were observed at 275 nm, 290 nm, 370 nm, 450 nm, and 505 nm. Among these observed peaks, while the ones at 275 nm and 290 nm generally represent amino acid tyrosine and tryptophan, respectively, the ones at 370 nm and 450 are coenzymes NADH and FAD, respectively. The peak at 505 nm corresponds to bile pigments. Compared to the controls, the relative intensity spectrum of samples with TT and TD was lower at 275 nm, 290 nm, and 370 nm, but higher at 450 nm and 505 nm.

The relative intensity ratio parameters R_1 and R_2 are shown in Fig. 2. The mean R_1 (I_{275}/I_{290}) values were 0.74 ± 0.07, 1.20 ± 0.14 and 1.33 ± 0.14 for the normal, TT and TD samples, respectively (Fig. 2a). The mean R_2 (I_{505}/I_{370}) values were 0.30 ± 0.20, 0.49 ± 0.52, and 22.40 ± 1.00 for the normal, TT and TD samples, respectively (Fig. 2b). Both the R_1 and R_2 values of the TT and TD samples were higher compared to those of the controls, though the

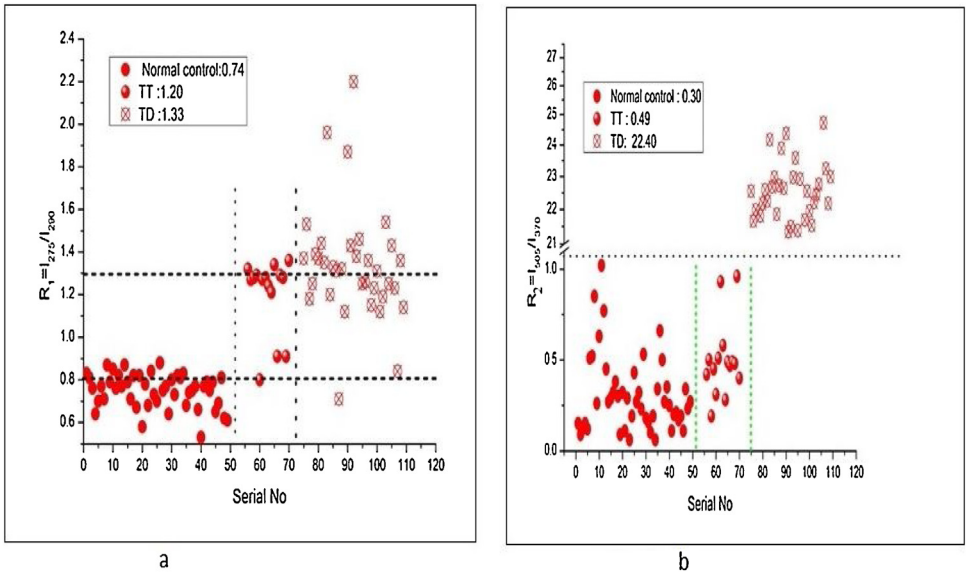


Fig. 2. Relative intensity ratios R1 and R2. Shown are the scatter plots for (a) R1 (I275/I290) and (b) R2 (I505/I370) of the control, TT and TD samples.

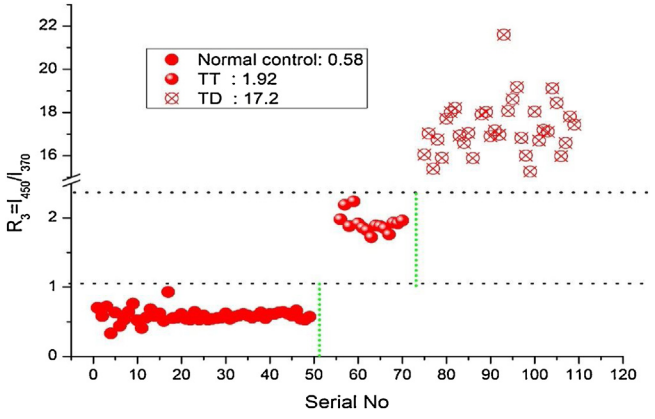


Fig. 3. Discrimination relative intensity ratio R_3. Shown are the R_3 (I_{405}/I_{370}) values of the control, TT and TD samples.

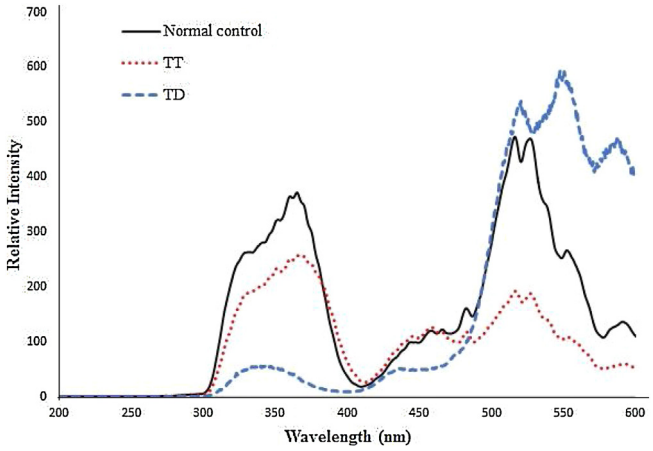


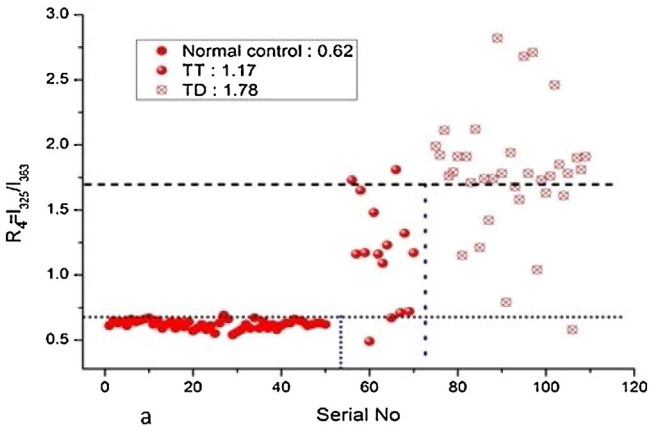
Fig. 4. Synchronous emission spectra with $\Delta\lambda = 10$ nm using plasma samples of normal controls, and patients with TT and TD.

mean value of the TT samples was closer to that of the controls. Fig. 3 shows that the relative intensity ratio R_3 for the analyzed samples. The mean R_3 (I_{450}/I_{370}) values were 0.58 ± 0.08, 1.92 ± 0.13, and 17.20 ± 1.29 for the normal, TT, and TD samples, respectively. The higher values in TD as compared to those in TT and controls indicate excessive hemolysis.

The synchronous emission spectra (SES) of control, TT, and TD blood plasma samples are shown in Fig. 4. The SES spectrum has main peaks at 325 nm, 363 nm, 460 nm, and 525 nm with different relative intensities. The relative intensities at 325 nm and 363 nm were lower in the TT and TD samples compared to those in the control samples. The peak at 525 nm was higher in the TD samples when compared to those in the TT and control samples. Two distinctive differences were observed in the spectrum in terms of peak proportion and relative intensity values. Fig. 5a shows the R_4 (I_{325}/I_{363}) values, which were 0.62 ± 0.03, 1.17 ± 0.07, and 1.78 ± 0.11 in the control, TT and TD samples. The values for the ratio parameter R_5 (I_{585}/I_{363}) were 0.38 ± 0.02, 1.83 ± 0.05, and 12.12 ± 0.08 for the control, TT, and TD samples, respectively. Both R_4 and R_5 values were higher for the TT and TD samples when compared to those of the control samples.

The fluorescence emission spectra(FES) spectrum of the acetone extracts of the cellular components of control, TT, and TD samples is shown in Fig. 6. The spectrum peak at 475 nm corresponds to the Raman band of acetone, while the two other major peaks at 585 nm and 635 nm represent the different forms of porphyrin, which are its basic and neutral forms. The overall relative intensity peaks in the TT and TD samples were lower compared those in the control samples. Fig. 7 shows the scatter plots for R_6 (I_{635}/I_{585}) for the control, TT, and TD samples. The spectral features of neutral and basic forms of porphyrins for normal control were 1.14 ± 0.05, 0.90 ± 0.04 for TT, and 0.52 ± 0.04 for TD. The R_6 values were lower in the TT and TD samples compared to the controls. While the R_6 of the TT samples was closer to that of the controls, that of the TD samples was 50% lower than the controls.

In the present study, the CBC was used to measure the blood parameters such as RBC counts. The mean RBC counts in the controls, and patients with TT and TD were 4.702×10^{12} /L, 4.02×10^{12}/L, and 2.98×10^{12}/L, respectively (Fig. 8). Compared to the controls, while the RBC counts in patients with TT were comparable, those in patients with TD were significantly lower. The mean Hb levels in the controls, and patients with TT, and TD were 135.5 g/L, 101.3 g/L, and 78.0 g/L, respectively (Fig. 9). Overall, when compared to the controls, while the Hb volume was lower in patients with TD, it was comparable in patients with TT.



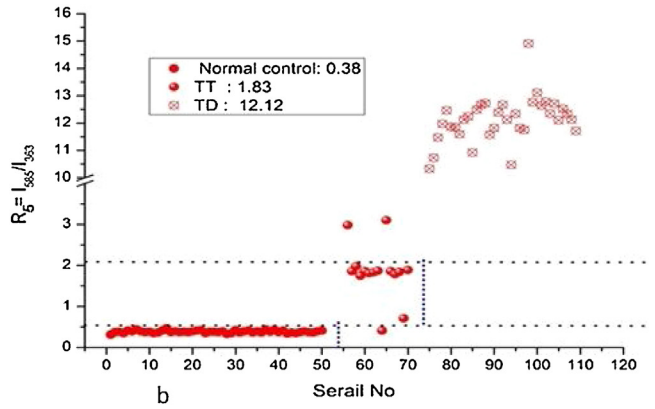


Fig. 5. Discrimination relative intensity ratios R_5 and R_6. Shown are the R_5 (I_{325}/I_{363}) and R_6 (I_{585}/I_{363}) values of the control, TT and TD samples.

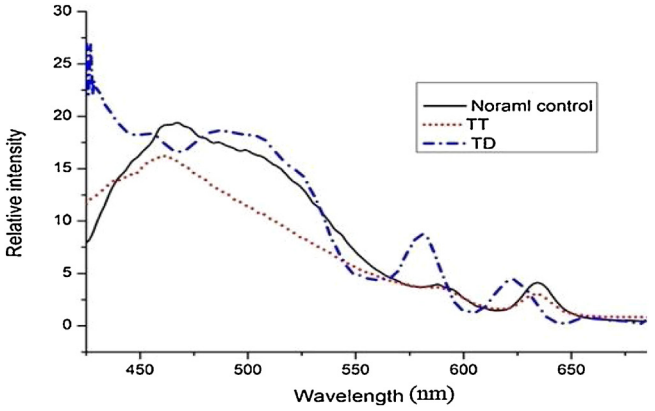


Fig. 6. Fluorescence emission spectrum of acetone extracts of the cellular components of control, TT and TD samples.

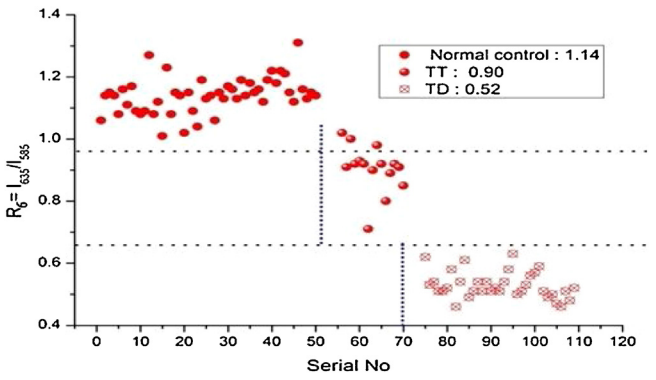


Fig. 7. Discrimination of the three sets of ratio parameters R_6 (I_{635}/I_{585})of the control, TT and TD samples.

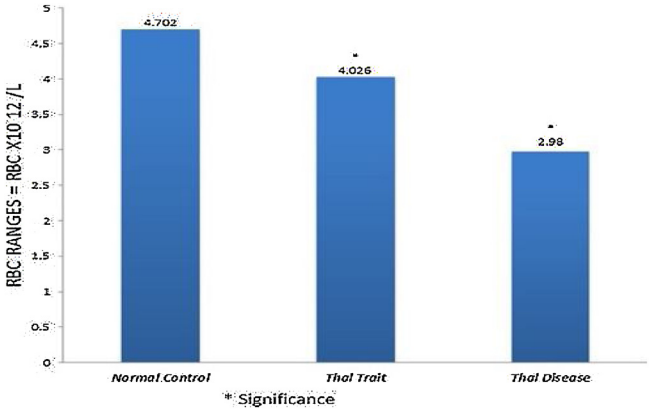


Fig. 8. Complete blood count images arethe average RBC counts in control, TT and TD samples.

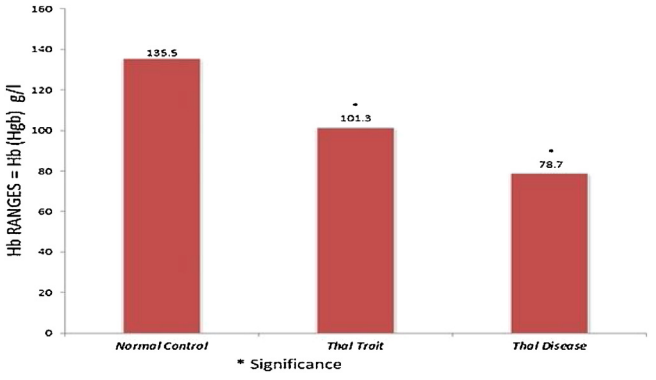


Fig. 9. Hemoglobin levels. The average hemoglobin values in control, TT and TD samples by CBC.

The Hb electrophoresis values in control, TT, and TD samples are shown in Fig. 10a–c. The HbA and HbA_2 values were 97.5% and 2.5%, respectively in the control, 96.1% and 3.9%, respectively in the TT and 94.5% and 5.5%, respectively in the TD samples. It was compared to the controls, the HbA values were lower in patients with TT and TD, whereas the HbA_2 values were higher in patients with TT (3.9%) and TD (5.5%). The average HbA values were 98.5%, 94.3%, and 78.6%, in the control, TT, and TD samples, respectively (Fig. 11). The HbA values were lower in both patients with TT and TD compared to the controls. The HbA volumes of the TT samples were comparable to those of the controls, while those of the TD samples were approximately 20%–30% lower. The mean HbA_2 values in the control, TT, and TD samples were 2.6.1%, 3.2%, and 6.8%, respectively (Fig. 12). The average values were higher in both TT and TD samples compared to those in the controls. The mean values of HbA_2 were two times higher in TD compared to those in the controls, while those in TT was comparable to the control values.

Discussion

These spectral findings were consistent with those of previous studies that used conventional methods and reported that the biomarkers tyrosine, tryptophan, NADH, and FAD are involved in many cellular redox activities [22–25]. In a previous study, the tyrosine levels in patients with thalassemia were lower compared to

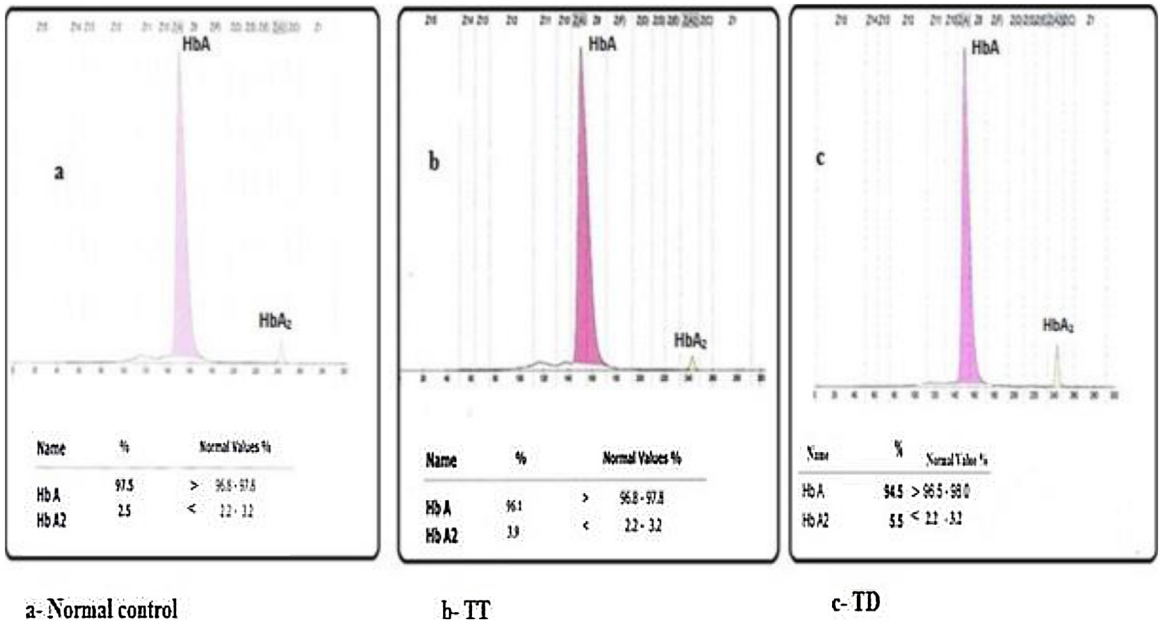


Fig. 10. Hemoglobin electrophoresis. Shown is a typical image of Hb- electrophoresis using (a) control, (b) TT and (c) TD samples.

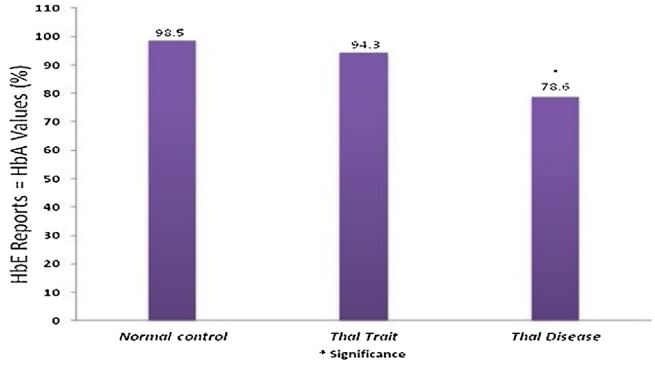


Fig. 11. Distribution of average values of HbA on normal control, TT and TD by Hb-electrophoresis.

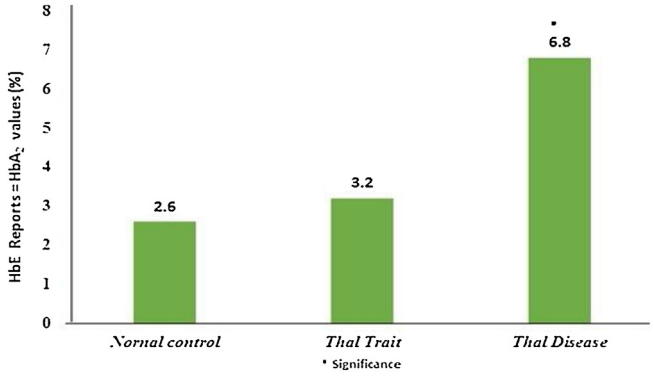


Fig. 12. Distribution of average values of HbA_2 in control, TT and TD samples by Hb-electrophoresis.

those in the control group [26]. Decreased m-tyrosine levels lead to oxidative damage in patients with thalassemia [27]. The low production and excessive damage of tryptophan lead to different types of anemia, including thalassemia [28]. The NADH interacts with RBCs to reduce the excessive hemolysis. NADH plays an important in the energy transfer in RBC and in the redox system. Reduction in the enzymes NADH and FAD leads to a lower production of RBCs, which causes anemia [29]. Consistent with other studies [18,19], our report exhibited that the SES and SXS spectrum were comparable. Moreover, we observed that the RBCs decay at a much faster rate in patients with thalassemia than in normal individuals. Earlier report showed the porphyrin levels in beta thalassemia patients were abnormally elevated and also patients with thalassemia, abnormal erythrocytes are produced in excessive numbers [30]. Another study reported that the porphyrin values in free erythrocytes could be useful in the diagnosis of anemia and in the determination and discrimination of its different stages [31].

Earlierreport, the CBC as a diagnostic modality for hemolytic TT reported that the mean values of blood parameters such RBC, Hb, and mean corpuscular hemoglobin were lower in the patients with TT compared to the control group [32]. Oxidative stress in erythrocytes increases the rate of cell decay in thalassemia, resulting in the insufficient supply of oxygen [33]. In a previous study, the average Hb level in normal controls was 139 g/L. In patients with TT and TD the mean Hb and HbA levels were 112 g/L, and 86 g/L, respectively [34]. Hb electrophoresis is useful in diagnosing thalassemia and sickle cell diseases based on other blood parameters, i.e., HbA, HbA_2, and HbS [35–37].

In previous studies, the mean value of HbA in the controls was 97%, while it was considerably lower in patients with thalassemia minor and major [38–40]. Furthermore the mean value of HbA_2 in the control group was 2.62. A mean value of above 3 and less than 5 indicates borderline disease, while a mean value above 5 indicates disease [38–40].

Conclusion

In this study, the patients with TT and TD were compared to normal individuals based on the levels of fluorescent biomolecules in their blood samples. Patients with thalassemia had significantly higher levels of the biomolecules tyrosine, tryptophan, NADH, FAD, and porphyrin, indicating excessive hemolysis. Furthermore, both CBC and Hb electrophoresis were used to differentiate between patients and the controls, and we demonstrate that the RBC, Hb, HbA, and HbA_2 levels were all higher in the patients. Similar results were achieved on spectral diagnosis with a high accuracy (above 95%). Based on the spectrum and relative intensity algorithms, the

spectral diagnosis could be used for thalassemia with an 85% accuracy for blinded groups. In summary, fluorescence spectroscopy is a simple and alternative method for premarital screening of thalassemia in remote areas.

Author contributions

SD, MSA, and VM designed the study, conducted the spectral analyses, interpreted reports and wrote the manuscript. FA provided the blood samples and helped the conventional result analyses. JP helped to improve the text.

Funding

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Competing interests

None declared.

Ethical approval

The research protocol was approved by the Institutional Review Board (Approval No.: E-17-2267).

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References

[1] Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood 2010;115(22):4331–6.
[2] Telen MJ, Kaufman RE. Normal hematologic system, the mature erythrocyte. Wintrobe's clinical hematology part II. Philadelphia, PA: Lippincott Williams & Wilkins; 2004. p. 217–48.
[3] WHO (World Health Organization). Sickle-cell disease and other haemoglobin disorders. Fact sheet No 308. WHO Media centre; 2011.
[4] Ibrahim NK, Bar H, Fakeeh A, Ahmadi J, Qadi M, Bar A, et al. An educational program about premarital screening for unmarried female students in King Abdul-Aziz University, Jeddah. J Infect Public Health 2011;4(1):30–40.
[5] Rachmilewitz EA, Giardina PJ. How I treat thalassemia. Blood 2011;118(13):3479–88.
[6] Dacie JV, Mollison PL, Richardson N, Selwyn JG, Shapiro L. Atypical congenital haemolytic anemia. Q J Med 1953;22(85):79–86.
[7] David HKC. α-Thalassemia: Hb H disease and HbBarts hydrops-fetalis. Ann N Y Acad Sci 2005;1054(1):25–32.
[8] Piel FB, Weatherall DJ. The alpha-thalassemias. N Engl J Med 2014;371(20):1908–16.
[9] Antonio C, Renzo G. Beta-thalassemia. Genet Med 2010;12(2):61–76.
[10] Wasi P. Haemoglobinopathies including thalassaemia. Part 1: Tropical Asia. Clin Haematol 1981;10(3):707–29.
[11] Vichinsky EP. Changing patterns of thalassemia worldwide. Ann N Y Acad Sci 2005;1054:18–24.
[12] Nienhuis AW, Nathan DG. Pathophysiology and clinical manifestations of the β-Thalassemias. Cold Spring Harb Perspect Med 2012;2(12):e011726.
[13] Eldanasoury AS, Boshnak NH, Abd El-Monem RE. Validation of criteria for smear review following automated blood cell analysis in ain shams university laboratory. Int J Sci Res 2016;5(3):484–93.
[14] Winichagoon P, Svasti S, Munkongdee T, Chaiya W, Boonmongkol P, Chantrakul N, et al. Rapid diagnosis of thalassemias and other hemoglobinopathies by capillary electrophoresis system. Transl Res 2008;152(4):178–84.
[15] Colah RB, Surve R, Sawant P, D'Souza E, Italia K, Phanasgaonkar S, et al. HPLC studies in Hemoglobinopathies. Indian J Pediatr 2007;74(7):657–62.
[16] Chen TP, Liu TC, Chang CS, Chang JG, Tsai HJ, Lin SF. PCR-based analysis of alpha-thalassemia in Southern Taiwan. Int J Hematol 2002;75(3):277–80.
[17] Masilamani V, Al-Zhrania K, Al-Salhia M, Al-Diabb A, Al-Ageily M. Cancer diagnosis by auto fluorescence of blood components. J Lumin 2004;109(3–4):143–54.
[18] Masilamani V, Vijmasi TA, Salhi M, Govindaraj K, Vijaya-Raghavan AP, Antonisamy B. Cancer detection by native fluorescence of urine. J Biomed Opt 2010;15(5):057003–9.
[19] AlSalhi MS, Algahtani FH, Devanesan S, Vijmasi VT, Jeyaprakash K, Alsaeed AH, et al. Spectral detection of thalassemia: a preliminary study. J Biomed Sci 2014;21(26):1–8.
[20] Serjeant BE, Forbes M, Williams LL, Serjeant GR. Screening cord bloods for detection of sickle cell disease in Jamaica. Clin Chem 1974;20(6):666–9.
[21] Devanesan S, AlSalhi MS, Ravikumar M, Perinbam K, Prasad S, Abbas HA, et al. Fluorescence spectral classification of iron deficiency anemia and thalassemia. J Biomed Opt 2014;19(2):027008–14.
[22] Lakowicz JR. Protein fluorescence, principles of fluorescence spectroscopy. Springer US; 2006. p. 529–75. SBN. 978-0-387-46312-4.
[23] Clark EH, East JM, Lee AG. The role of tryptophan residues in an integral membrane protein: diacylglycerol kinase. Biochemistry 2003;42(37):11065–73.
[24] Ladokhin AS, Jayasinghe S, White SH. How to measure and analyze tryptophan fluorescence in membranes properly, and why bother. Anal Biochem 2000;285(2):235–45.
[25] Kilbridge TMW, Fried PH. The mechanism by which plethora suppresses erythropoiesis. Blood 1969;33(1):104–13.
[26] Abdulrazzaq YM, Ibrahim A, Al-Khayat AI, Dawson K. Beta-thalassemia major and its effect on amino acid metabolism and growth in patients in the United Arab Emirates. ClinChimActa 2005;352(1–2):183–90.
[27] Matayatsuk C, Poljak A, Bustamante S, Smythe GA, Kalpravidh RW, Sirankapracha P, et al. Quantitative determination of ortho- and meta-tyrosine as biomarkers of protein oxidative damage in β-thalassemia. Redox Rep 2007;12(5):219–28.
[28] Hankes LV, Brown RR, Schiffer L, Schmaeler M. Tryptophan metabolism in humans with various types of anemias. Blood 1968;32(4):649–61.
[29] Kuhn V, Diederich L, Keller TCS, Kramer CM, Lückstädt W, Panknin C, et al. Red blood cell function and dysfunction: redox regulation, nitric oxide metabolism, anemia. Antioxid Redox Signal 2017;26(3):718–42.
[30] Stockman JA, Weiner LS, Simon GE, Stuart MJ, Oski FA. The measurement of free erythrocyte porphyrin (FEP) as a simple means of distinguishing iron deficiency from beta-thalassemia trait in subjects with microcytosis. J Lab Clin Med 1975;85(1):113–9.
[31] Baytaeva DA, Bessmeltsev SS. The metabolitic role of the iron porphyrin complex in the development of anemic syndrome in patients with minor form of β-thalassemia. Kazan Med J 2012;93(1):7–11.
[32] Pengon J, Svasti S, Kamchonwongpaisan S, Vattanaviboon P. Hematological parameters and red blood cell morphological abnormality of Glucose-6-Phosphate dehydrogenase deficiency co-inherited with thalassemia. Hematol Oncol Stem Cell Ther 2018;11(1):18–24.
[33] Olivieri O, De Franceschi L, Capellini MD, Girelli D, Corrocher R, Brugnara C. Oxidative damage and erythrocyte membrane transport abnormalities in thalassemias. Blood 1994;84(1):315–20.
[34] Khosa SM, Usman M, Moinuddin M, Mehmood HO, Qamar K. Comparative analysis of cellulose acetate hemoglobin electrophoresis and high performance liquid chromatography for quantitative determination of hemoglobin A2. Blood Res 2015;50(1):46–50.
[35] Fucharoen S, Winichagoon P, Wisedpanichkij R, Sae-Ngow B, Sriphanich R, Oncoung W, et al. Prenatal and postnatal diagnoses of thalassemias and hemoglobinopathies by HPLC. Clin Chem 1998;44(4):740–8.
[36] Vincent O, Oluwaseyi B, James B, Saidat L. Coinheritance of b-thalassemia and sickle cell anaemia in southwestern Nigeria. Ethiop J Health Sci 2016;26(6):517–22.
[37] Galanello R. Screening and diagnosis for haemoglobin disorders. Principles. In: Prevention of thalassaemias and other haemoglobin disorders. 2nd ed; 2014. Chapter 4 (1).
[38] Yang Z, Chaffin CH, Easley PL, Thigpen B, Reddy VV. Prevalence of elevated hemoglobin A2 measured by the CAPILLARYS system. Am J ClinPathol 2009;131(1):42–8.
[39] Elsayid M, Al-Shehri MJ, Alkulaibi YA, Alanazi A, Qureshi S. Frequency distribution of sickle cell anemia, sickle cell trait and sickle/beta-thalassemia among anemic patients in Saudi Arabia. J Nat Sci Biol Med 2015;6(Suppl. 1):S85–8.
[40] Velasco-Rodríguez D, Blas C, Alonso-Domínguez JM, Vega G, Soto C, García-Raso A. Cut-Off values of hematologic parameters to predict the number of alpha genes deleted in subjects with deletional alpha thalassemia. Int J Mol Sci 2017;18(12):e2707.