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DIAGNOSIS OF β-THALASSEMIA TRAIT (βTT) IN IRAQI PATIENTS: HEMOGLOBIN A_2 (HbA_2) AND BIOCHEMICAL APPRAISAL

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ABSTRACT

The most frequent forms of α- and β-degenerate anaemias are characterized by disruption in the formation of bead protein which leads to the defective hemoglobin condition called thalassemia, a prevalent hereditary blood illness. The present study was aimed to diagnose haemoglobin A2 (HbA2) in patients suffering from thalassemia so as to provide a reference for further improvements in thalassemia control. Data was collected from 155 thalassemia patients at Baghdad Medical City between November 2024 and March 2025 regarding red blood cell (RBC), hemoglobin (Hb), mean erythrocyte volume (MCV), mean corpuscular hemoglobin (MCH), hemoglobin (HbA), hemoglobin A2, and fetal hemoglobin (HbF). The relationships between different parameters were analysed. HPLC results demonstrated that thalassemia patients had significantly higher mean for HBA2, HBA, and HBF (P≤0.01) in comparison to the control group. The correlation coefficient results between serum ferritin and RBC in HBA2 patient group were insignificant (P≤0.01). The study revealed that the individuals exhibiting β-thalassemia trait have normal levels of serum ferritin, slightly elevated red blood cell counts, and high levels of HBA2.

Keywords: β-thalassemia, complete blood count, hemoglobin A2 (HbA2), serum ferritin

INTRODUCTION

Thalassemia is a significant public health concern and most common in Southeast Asia, India, and the Mediterranean region. The patients with β-thalassemia major have severe chronic hemolytic anaemia and require lifelong blood transfusions (Rao *et al.*, 2024; Jarujareet *et al.*, 2025). Thalassemia is categorized into three types: alpha (α), beta (β), and delta-beta (δβ); amongst which β-thalassemia is the most prevalent. Worldwide, of the 5% patients 3% fall in β-thalassemia category (Begum *et al.*, 2024). Iron chelation therapy and systematic red blood cell (RBC) transfusions are most often used supportive treatments for thalassemia, and these treatments have significantly increased the patient's life span. Regardless, endocrine disorders connected to β-thalassemia can still develop, and the most common symptoms include hypoxia, iron overload, and chronic anaemia (Venou *et al.*, 2024). Evidences suggest that thalassemia was more prevalent in tropical and subtropical regions where malaria was extremely prevalent (Origa, 2017; Qian *et al*., 2025). The symptoms observed in patients

suffering β-thalassemia intermedia are similar to those of both carriers and significant cases of the disease. Ineffective erythropoiesis and hemolysis can cause morbidity, like ulcers, pulmonary hypertension, and discomfort, even in patients with moderate anaemia (Kattamis *et al.,* 2020). The Mediterranean, the Middle East, and Southeast Asian regions have historically the highest β-thalassemia prevalence, whereas Northern Europe and North America have the lowest (Mohanty *et al.,* 2013; Kattamis *et al.,* 2020). In Middle East, for example, the disease has long been common among the native populations owing to high rates of carrier status and consanguineous marriages, as well as the large migration of people from other regions with high rates of disease prevalence, including Southeast Asia (El-Beshlawy *et al.,* 2024). Decreased δ globin chain synthesis can lead to a drop in HbA2 levels. δ globin chain synthesis may be induced by transcriptional oversights or, in rare cases, by post-translational alterations in HbA2 tetramer assembly. A crucial step in the creation of Hb tetramer is the dimerization of charged α- and non-α subunits (Colaco and Nadkarni, 2021). There has been an increase in prenatal screening with complete blood count (CBC) that includes red blood cell indices such MCV, MCH, and RBC count. Poor nutrition, worm infestation, and numerous childbirths cause iron deficiency anaemia, which hinders the utilization of this approach. Gold standard test, a combination of CBC and high-performance liquid chromatography (HPLC), is frequently used to screen thalassemia. The hemoglobinopathies have been linked to around 200 distinct mutations. Nevertheless, 93.6% of all mutations are accounted for the five most prevalent mutations (Qian *et al.,* 2025). The present study was aimed to study the diagnostic value of HbA2 in patients suffering from thalassemia, as well as to provide a reference for further improvements in thalassemia prevention and control as well as clinical consultation in this region by analysing the results of haematological characteristics of Iraqi patients.

MATERIALS AND METHODS

Study design and participants

The present study involved two groups of patients. The first group included 155 patients (60 men and 95 females) with confirmed β-thalassemia trait (βTT) who attended the Baghdad Medical City (BMC), Iraq from November 2024 to March 2025. Only the patients with a confirmed diagnosis of β-thalassemia trait were chosen for this group and excluded those with other kinds of anaemia or receiving therapy for anaemia. The second group (control group) comprised of 50 apparently healthy adults (25 men and 25 women). Anticoagulated peripheral blood (2 mL) was collected from each patient using EDTA tubes for automated haematological analysis (SYSTEM XN-350). Serum ferritin was measured in simple tubes using an automatic immunoassay analyser (AIA-2000 System, Tosoh Corporation, Japan). Haemoglobin fractions were determined using high-performance liquid chromatography (HPLC) system (model: H9SX30010, Tosoh Corporation, Japan). Only those βTT subjects were chosen in this study who had HbA2 > 3.5%, mean corpuscular value (MCV) < 80 fL, mean corpuscular haemoglobin (MCH) < 26 pg, red blood cell (RBC) count > 4.5 million cells μL^{-1} in males and > 3.8 million cells μL^{-1} in females, and serum ferritin > 30 ng mL^{-1} [to rule out any iron deficiency]. The increase in HbA2 levels (>3.5%) is considered the most significant variable in detecting β-thalassemia carriers.

Statistical analysis

The Statistical Analysis System - SAS (2018) program was used to determine the effect of different groups on the studied parameters. The t-test was performed to find significant differences between the groups, if any. In this study, correlation coefficient between the HBA2 patient group and serum ferritin and RBC was also worked out (Marasinghe and Koehler, 2018).

RESULTS AND DISCUSSION

A total of 234 patients were screened at BMC, Bagdad (Iraq) between November 2024 to March 2025, and out of these 155 patients had βTT (60 males, 95 females) by confirmation through HPLC. The patients exhibited mean haemoglobin, RBC count, MCV, MCH, and serum ferritin values of 10.00 ± 0.29 g dL^{-1}, 5.078 ± 0.11 million cm^{-3}, 62.05 ± 1.21 fL, 19.40 ± 0.43 pg and 80.47 ± 14.69 µg dL^{-1}, respectively. These values were significantly lower than the control haemoglobin ($p \leq 0.01$), non-significant with serum ferritin control (99.08 ± 10.40 µg dL^{-1}), and highly significant with RBC control (4.71 ± 0.12 million cm^{-3}, $p \leq 0.05$) [Table 1]. The HPLC results of patients showed that the mean values for HBA2, HBA, and HBF were 4.49 ± 0.08, 77.26 ± 1.03 and 3.23 ± 0.30%, respectively. These values were significantly higher than control ($p < 0.01$). While the control levels of HBA2, HBA, and HBF were 2.32 ± 0.12, 93.76 ± 0.64, and 1.81 ± 0.33%, respectively (Table 2).

Table 1: Comparison of β-thalassemia trait (βTT) patients and control groups with respect to the various haematological parameters (mean ± SE)

Groups	Serum ferritin (ng mL^{-1})	Hemoglobin (g L^{-1})	Red blood cells (million μL^{-1})	MCV (fL)	MCH (pg)
βTT patients	80.47 ± 14.69	10.00 ± 0.29	5.078 ± 0.11	62.05 ± 1.21	19.40 ± 0.43
Control	99.08 ± 10.40	13.96 ± 0.28	4.710 ± 0.12	88.69 ± 1.09	30.39 ± 0.54
t-test	44.635 (ns)	0.929**	0.267*	3.798**	1.446**
p-value	0.405	0.0001	0.050	0.0001	0.0001

*(p≤0.05), **(p≤0.01); ns = Non-significant; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin

Table 2: Comparison between βTT patients and control groups for HBA2, HBA and HBF (mean ± SE)

Groups	HBA2 %	HBA %	HBF %
βTT patients	4.49 ± 0.08	77.26 ± 1.03	3.23 ± 0.30
Control	2.32 ± 0.12	93.76 ± 0.64	1.81 ± 0.33
t-test	0.296**	3.091**	0.992**
p-value	0.0001	0.0001	0.0060
** $p \leq 0.01$			

Among the many hereditary disorders, thalassemia is the more prevalent monogenic form. Every year, hundreds of thousands of children are born with thalassemia major, and over 300 million people around the world carry the thalassemia gene. This puts a heavy burden on families and communities. Many thalassemia cases occur in Asia and the Mediterranean (Qian *et al.*, 2025). In North Eastern region of Iraq 56.1% families with milder thalassemia phenotypes have a β0-thal mutation, according to the largest study on β-TI (Amin *et al.*, 2020). Therefore, it is wise to evaluate genetic modifiers of disease severity in β0 homozygous or compound heterozygous individuals. In addition, due to the restricted transfusion and chelation therapy, bone disorders affected slightly more than half of the β-TI patients (Amin *et al.*, 2020). The studies have revealed that β-thalassemia mutations in Iraq's capital are diverse and found multiple preventative markers (Hassan *et al.*, 2024). The possible causes for Iraq's elevated thalassemia rate include the country's weak laws, its extensive practice of consanguineous marriages, and the absence of adequate prophylactic initiatives. As per the Iraq family health survey nearly two-thirds of all marriages in Iraq's southern and central governorates and nearly half of all marriages in Iraq's northern and Kurdistan governorates are between relatives (Kadhim *et al.*, 2017). The Mentzer index and other cell-count-based indices were found reliable and conveniently accessible for detecting βTT in the study. β-thalassemia trait can be distinguished from other disorders by a high RBC count and a low MCV (Brancaleoni *et al.*, 2016; Jameel *et al.*, 2017). We excluded iron deficiency anaemic (IDA) patients by using S-ferritin in our investigation.

The patient group had significantly higher mean RBC level as compared to the control group ($p \leq 0.01$), with mean ± SD of many hemostatic parameters measured in people with microcytic and/or hypochromic anemia. Also highlighted are the results of using each of the six different prediction formula. The level of hemoglobin A2 (HBA2) did not differ significantly ($p < 0.05$) as per t-test.

Table 3: Effect of gender on HBA2 of Iraqi patients

Parameter	Means ± SE		P-value
	Male	Female	
HBA2%	4.45 ± 0.17	4.52 ± 0.09	0.702 (ns)

Table 4: Correlation coefficient between HBA2 and serum ferritin and RBC count in Iraqi patients

Parameters	Correlation coefficient (r value)	p-value
HBA2 & S-ferritin	-0.09 (ns)	0.6057
HBA2 & RBC	0.16 (ns)	0.4081

NS: Non-significant

The present study found no significant correlation coefficient (r) between serum ferritin and RBC in HBA2 patient group (Table 4). The results of other tests, like HB, MCV, and MCH have been disregarded since they are often mistaken for indicators of other diseases, such as IDA. The diagnostic measure HbA2 is the key significant parameter for βTT screening **(**Mosca *et al.*, 2009; Gao and Liu, 2022). When it comes to measuring HbA2, most clinical laboratories depend on HPLC (Anandani *et al.*, 2025); so was a preferred strategy in this study. Our study found that compared to the control group, βTT patients had significantly higher levels of HBA2 and RBC count, while serum ferritin was not significantly different (normal). This study identifies the factors that contribute to the borderline HbA2 levels, examined the impact of confounding factors on HbA2 and MCV levels, and interprets the results for β-thalassemia carrier screening.

There was no significant difference in HbA2 levels between males and females among the patients with β-thalassemia phenotype. It appears that gender does not significantly affect HbA2 concentrations in people with this hereditary disorder (Obiorah *et al.*, 2025; Zhang *et al.*, 2025). In patients with β-thalassemia trait no correlation was observed between serum ferritin, RBC, and HBA2 (Table 4). The fact that HbA2 levels are same in men and women provide credence to the idea that heredity, not hormones or physiology, is the primary determinant of its expression. Gaining a grasp of this consistency can help in precise identification and categorization of β-thalassemia trait, guaranteeing that changes in HbA2 levels are understood correctly in medical evaluations (Obiorah *et al.*, 2025). This study suggests that β-thalassemia trait can be accurately diagnosed. Due to the limited sample size and deletion of certain red cell indices, the current study has certain limitations and the future research should assess the discriminatory power of all already published red cell indices.

Conclusion: The study revealed that β-thalassemia trait patients usually have normal serum ferritin levels, slightly higher RBC counts, and high HBA2 levels. Screening the βTT suspect individuals for HBA2, RBC count, and serum ferritin seems to be a dependable and efficient index. Early carrier screening, as well as intensive premarital screening and counselling programs, combined with strong legislation, can help to reduce the prevalence of β-thalassemia major.

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Ethical statement: Since this study used the routinely obtained laboratory data and did not involve any intervention or direct patient contact, so no ethical approval was required.

Conflict of interest: There are no conflicts of interest to declare regarding the publication of this research apart from those disclosed**.**

REFERENCES

Amin, S.S., Jalal, S.D., Ali, K.M., Mohammed, A.I., Rasool, L.K. and Osman, T.J. 2020. Beta thalassemia intermedia: A single thalassemia center experience from Northeastern Iraq. *BioMed Research International*, **2020**(1): 2807120. [*https://doi.org/10.1155/2020/2807120*].

Anandani, G., Motiani, A., Goswami, P. and Sonagra, A. 2025. Challenges associated with the

identification of abnormal hemoglobin variants utilizing the high-performance liquid chromatograph technique: A prospective study in a hospital setting in Gujarat. *International Journal of Applied and Basic Medical Research*, ***15***(3): 197-205.

Begum, R., Suryanarayana, G., Rama, B.S. and Swapna, N. 2024. An overview of thalassemia: A review work. *Artificial Intelligence, Blockchain, Computing and Security*, **1:** 796-804.

Brancaleoni, V., Di Pierro, E., Motta, I. and Cappellini, M.D., 2016. Laboratory diagnosis of thalassemia. *International Journal of Laboratory Hematology*, **38**: 32-40.

Colaco, S. and Nadkarni, A. 2021. Borderline HbA2 levels: Dilemma in diagnosis of beta-thalassemia carriers. *Mutation Research/Reviews in Mutation Research*, **788**: p.108387. [*https://doi.org/10.1016/j.mrrev.2021.108387]*.

El-Beshlawy, A., Dewedar, H., Hindawi, S., Alkindi, S., Tantawy, A.A., Yassin, M.A., *et al.*, 2024. Management of transfusion-dependent β-thalassemia (TDT): Expert insights and practical overview from the Middle East. *Blood Reviews*, **63**: p. 101138. [*https://doi.org/10.1016/j.blre.2023.101138*].

Gao, J. and Liu, W. 2022. Advances in screening of thalassaemia. *Clinica Chimica Acta*, **534**: 176-184.

Hassan, M.K., Abbas, R.A., Hassan, R.A., Taghlubee, I.M., Abd Al Majeed, S.S., Khaleel, G.A., *et al.*, 2024. Prevalence and spectrum of β-thalassemia mutations in Baghdad, Iraq: Data from the premarital screening program. *Hemoglobin*, **49**(1): 31-37.

Jarujareet, U., Wiratchawa, K., Petiwathayakorn, T., Koonyosying, P., Hantrakool, S., Srichairatanakool, S. *et al*., 2025. Classification of beta-thalassemia major and HbE/beta-thalassemia via deep learning of image structure function image. *Biomedical Signal Processing and Control*, **102**: p. 107265. [*https://doi.org/10.1016/j.bspc.2024.107265*].

Kadhim, K.A., Baldawi, K.H. and Lami, F.H. 2017. Prevalence, incidence, trend and complications of thalassemia in Iraq. *Hemoglobin*, **41**(3): 164-168.

Kattamis, A., Forni, G.L., Aydinok, Y. and Viprakasit, V. 2020. Changing patterns in the epidemiology of β-thalassemia. *European Journal of Haematology*, **105**(6): 692-703.

Marasinghe, M.G. and Koehler, K.J. 2018. Introduction to the SAS language. pp. 1-68. **In**: *Statistical Data Analysis Using SAS: Intermediate Statistical Methods*. Springer International Publishing, [*https://doi.org/10.1007/978-3-319-69239-5_1*].

Mohanty, D., Colah, R.B., Gorakshakar, A.C., Patel, R.Z., Master, D.C., Mahanta, J., *et al.*, 2013. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: A multicentre study. *Journal of Community Genetics*, **4**: 33-42.

Mosca, A., Paleari, R., Ivaldi, G., Galanello, R. and Giordano, P.C. 2009. The role of haemoglobin A2 testing in the diagnosis of thalassaemias and related haemoglobinopathies. *Journal of Clinical Pathology*, **62**(1): 13-17.

Obiorah, I., McCall, C.M., Balmaceda, A., Salansky, S., Agarwal, A. and Pozdnyakova, O. 2025. An 18-year review of hemoglobinopathy proficiency testing: Recommendations from the College of American Pathologists, Hematology and Clinical Microscopy Committee. *Archives of Pathology & Laboratory Medicine*, **149**(9): 805-811.

Origa, R., 2017. β-Thalassemia. *Genetics in Medicine*, **19**(6): 609-619.

Qian, H., Zhao, W., Li, W., Wu, Y., Lin, X., Huang, J., *et al.*, 2025. Analysis of thalassemia genotypes and HbA2 test results in pregnant women in Shenzhen, China. *Scientific Reports*, **15**(1): p. 7483. [*https://www.nature.com/articles/s41598-025-91908-x*].

Rao, E., Chandraker, S.K., Singh, M.M. and Kumar, R. 2024. Global distribution of β-thalassemia mutations: An update. *Gene*, **896**: p.148022. [*DOI: 10.1016/j.gene.2023.148022*].

Venou, T.M., Barmpageorgopoulou, F., Peppa, M. and Vlachaki, E. 2024. Endocrinopathies in beta thalassemia: A narrative review. *Hormones*, **23**(2): 205-216.

Zhang, Y., Wu, J., Ren, L., Li, F., Wu, X., Guo, M., *et al*., 2025. Large-scale analysis of the thalassemia mutation spectrum in Guizhou province, Southern China, using third-generation sequencing. *Clinical Genetics*. **108** (2): 156-167.