ORIGINAL ARTICLE

The Impact of *BCL11A* Variants and Immune Factors on β-Thalassemia Major in Iraqi

Orass M.S. Al-Taei*
Department of Microbiology, College of Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq

Key words:
β-TM, BCL11A gene, complement factors, antibodies, polymorphism

Corresponding Author:
Orass Madhi Shaheed - Department of Microbiology, College of Medicine, University of Al-Qadisiyah
Tel: 0096407802250803
orass.shaheed@qu.edu.iq
Authors ORCID:
0000-0002-6009-6847

ABSTRACT

Background: *β-Thalassemia major (β-TM) is a significant health problem in Iraq. Therefore, more studies and scientific research are needed to find appropriate solutions to this problem.* ***Objectives:*** *The existing research aims to determine the role of the polymorphism of the BCL11A (rs11886868) gene and evaluate its relationship with the development of β-TM severity and fetal hemoglobin (HbF) level in addition to appraising the character of complement factors and antibodies in the pathogenesis of β-TM in the Iraqi population.* ***Methodology:*** *Before receiving a blood transfusion, 45 patients with β-TM underwent clinical evaluations and blood samples were collected. High-performance liquid chromatography was used to quantitatively measure HbF, and polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) was used to analyze the single nucleotide polymorphism rs11886868 in the BCL11A gene. Moreover, enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies and complement factors.* ***Results:*** *The current study showed that most children (56%) with thalassemia stood in the age group 9-14 years, and the highest percentage of infections was among females (58%).The immunological results showed that C3, C4, IgA, IgG, and IgM levels were elevated in β-TM patients (119.2 mg/ml,49.8 mg/ml, 292.2 mg/dl, 1851.2 mg/dl, and 242.11 mg/dl, respectively) compared HbF appeared to be elevated in β-TM patients (16.87 g/dl) compared with the healthy controls (1.73 g/dl).* ***Conclusion:*** *This study showed important alterations ($p<0.05$) in the distribution of mutant alleles, antibodies, complement factors, and HbF between patients and controls.*

INTRODUCTION

β-Thalassemia major is a congenital hemolytic anemia that represents a major and serious health problem and has the highest prevalence and incidence rates worldwide[1,2]. It results from mutations in β-globin genes, leading to defective synthesis of adult hemoglobin (Hb A). To date, over 200 mutations have been identified in β-globin genes that cause this illness[3,4]. Other genetic factors that map outside the β-globin gene cluster may also co-inherit and alter the clinical phenotypes of β-thalassemia. These factors have been proposed to determine the perseverance of HbF construction, which could potentially improve the clinical and haematological strictness of β-thalassemia[5]. Previous studies have detected a locus (rs11886868) on chromosome 2, located in the BCL11A.

B-cell lymphoma/leukaemia 11A gene, that is correlated with Hb F[6]. *The BCL11A gene encodes* a zinc-finger protein that inhibits γ-globin gene expression. Hence, it inhibits γ-globin chain synthesis and helps exchange Hb F for Hb A[7]. However, a single nucleotide polymorphism (SNP), rs11886868, in *BCL11A* has been associated with the upregulation of fetal Hb production[8]. Other studies have not been able to establish a relationship between this SNP and elevated HbF ranks in some ethnic groups, suggesting differences across populations[9].

Iraq and the Eastern Mediterranean region are affected by this disorder. It causes a range of clinical symptoms, from thalassemia minor in those who are heterozygous for it to β-TM in those who are homozygous or complex heterozygous for β-thalassemia mutations[10,11]. The β-TM phenotype is a severe disorder linked to numerous complications and a lifelong need for transfusions. These include liver disease, diabetes mellitus, hypothyroidism, hypoparathyroidism, hypogonadism, and stunted growth. With a significantly higher eminence of life and fewer difficulties in developed nations compared to developing nations, the frequencies of these complications vary somewhat among β-TM cohorts and are correlated with the effectiveness of the management and follow-up given to these patients [12, 13].

In addition to severe anemia, patients with beta-thalassemia major experience several other issues, such as heightened vulnerability to bacterial infections, which significantly contribute to their morbidity and mortality[14]. Numerous investigations have been conducted to assess potential immune system alterations in patients with thalassemia, considering both the humoral and cellular immune systems; however, no

reliable impairment in white blood cells or immune function has been reported to date[15,16] Previous studies have documented several immunological abnormalities, including increased serum immunoglobulin levels, changes in B- and T-cell numbers and function, and decreased opsonization and granulocyte phagocytosis[17] . The immune system may be severely harmed by several factors, including splenectomy, iron overload, repeated exposure to foreign antigens during blood transfusions, and the use of chelating agents[18]. This study aims to consider the role of the SNP rs11886868/ *BCL11A* polymorphism, HbF, immunoglobulins, and complement factors in the clinical development of Iraqi patients with β-TM**.**

METHODOLOGY

Study design and samples collection

Blood samples were taken as part of the current case-control investigation from 45 individuals suffering from β-TM from the Al- thalassemia center in Al-Diwaniyah province, and 45 samples were collected from healthy individuals as a control group during the period from 1/10/2023 to 5/4/2024, where the cases were diagnosed clinically and in the laboratory by specialist doctors. Consent was obtained from all participants when collecting the questionnaire and samples. The participants were aged 5–14 years and belonged to the same Arab race, in line with the population of Al-Diwaniyah province.

Ethical approval:

The study was approved by the Research Ethical Committees of Al-Qadisiyah University (no.30\5137 in 19\11\2023). Informed consent was obtained from all participants and/or their legal guardians.

Molecular study:

Genomic DNA was removed from whole blood samples according to the manufacturer's instructions for the Genomic DNA Mini Kit (Geneaid). PCR was used to amplify rs11886868 SNP in the *BCL11A* gene using a Professional TR/O Thermocycler (USA) with the primers 5'-TTTGGTGCTACCCTGAAAGAC-3' and 5'-ACTCAACAGTAGCAGAATGAA AGAG-3. *BCL11A/* rs11886868 SNP gene (T→C) polymorphism was identified using the RFLP method. The 548-bp PCR product was processed using MboII limit enzymes at 37°C for five min than analyzed by 2% agarose gel electrophoresis. The T allele lacks the MboII restriction site, whereas the C allele has it. The C allele was identified as two fragments of 470 and 70 bp[١٩,٢٠] .

Immunological study:

Serum levels of complement factors C3 and C4, in addition to antibodies IgG, IgM, and IgA, were evaluated using ELISA. LS-F4381 is a 96-well ELISA kit utilized for the quantitative detection of complement factors, whereas RayBiotech's ELISA kits were used to evaluate antibodies.

Statistical analysis

The statistical analysis was performed using SSPS (v. 20) and Excel 2010. The approximate percentage, mean, and Standard Deviation (SD) are dependent on the correlation between data by probability value that is significant at a level less than 0.05. When the test statistic is chi-squared distributed under the null hypothesis, the chi-squared test (also known as the $\chi 2$ test or Pearson's chi-squared test) is a valid statistical hypothesis test. Genotypes and allelic frequencies were calculated using the Hardy–Weinberg equation 21.

RESULTS

The existing research is a case – control study that comprised 45 patients with β-TM, aged 5–14 years, with an average age of 8.51 years and a standard deviation of ±1.02. The control group included healthy individuals aged between 5 and 14 years, with mean agean of 9.91 years and a standard deviation of 2.11. When comparing the mean age of patients with healthy controls, we did not observe any significant differences (P = 0.801), as shown in table (1). Most of the β-TM cases were in females, with a rate of 58%. The patients were divided into two age groups: 5–9 years (20 patients) and 9–14 years (25 patients). In contrast, all β-TM patients were separated into mild, moderate, and severe categories based on the strictness score, which was determined by the patients' Hb levels, age at onset, age at first blood transfusion, level of growth obstruction, and splenectomy. The results showed that 40% of the β-TM cases were moderate, 35% were severe, and 25% were mild, as shown in Figure 1.

Table 1: Demographical properties of β -TM patients and healthy individuals

Demographical	β-TM (cases)	Healthy individuals (control)	P value
Age	5-14	5-1	
Mean	8.51	9.91	0.801
SD	1.02	2.11	
SE	0.152	0.315	
Age groups	N (%)	N (%)	P value
5	20	22	0.665
9 - 1	25	23	666
Gender	N (%)	N (%)	
Females	26	21	51
Males	19	24	0.05
Total number	45	45	

Standard Deviation (SD), Standard Error (SE)

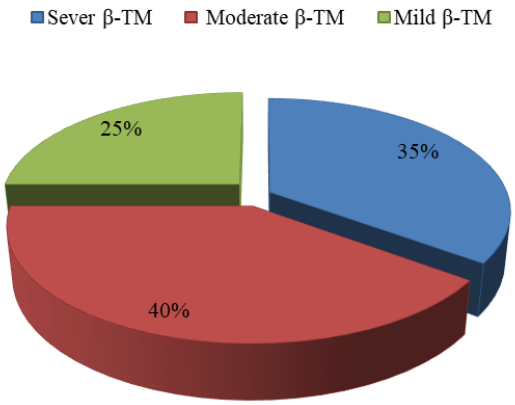

Fig. 1: Distribution cases according to severity of β – TM

According to Splenectomy, the results of the current search showed that 20 of 45 patients were splenectomized, and 40% of them were female, while the other cases were not splenectomized (25/45), and most of them were also female (56%). These differences in sex and splenectomy led to the emergence of significant differences, where the probability was < 0.05, as shown in table 2.

The immunological results showed that the serum concentrations of C3, C4, IgA, IgG, and IgM were elevated in β-TM patients (119.2 mg/ml,49.8 mg/ml, 292.2 mg/dl, 1851.2 mg/dl, and 242.11 mg/dl, respectively) compared with the control (36.7 mg/ml, 18.22 mg/ml, 183 mg/dl, 501.3 mg/dl, 37.4 mg/dl, and 1.73 mg/dl, respectively), and these differences were accompanied by significant differences where the probability value was less than 0.05, as shown in table 3 .In addition, the abnormal hemoglobin HbF appeared to be elevated in patients with β-TM (16.87 g/dl) compared with healthy controls (1.73 g/dl).

Table 2: Distribution of gender according to splenectomy

Splenectomy	Total	Males N	Female N (%)	P value
Splenectomised	20	8	12	0.02
Non-	25	11	14	0.0498
Total number	45	19	26	

The outcomes of the existing research showed that the immunological variables and HbF increase with blood transfusion, as their concentration in the serum of the splenectomized βTM patients was 128.6 mg/ml, 53.2 mg/mml 291.1 mg/dl,1868.3 mg/dl, 262.1 mg/dl,18.22 g/dl for each of C3, C4, IgA, IgG, IgM, and HbF, respectively, while it appeared lower in the non-splenectomized βTM group as follows: 118.4 mg/dl, 47.3 mg/dl, 200 mg/dl, 1722.6 mg/dl, 204.8 mg/dl, and 14.16 g/dl, respectively, as shown in table 4.

Table 3: Case – control appraisal serum level of antibodies and complement factors

Biological markers	Cases	Control	P value
Complement factors	Mean ± SD	Mean ± SD	
C3 (mg/ml)	119.2 ± 56.39	36.7 ± 8.93	0.009*
C4 (mg/ml)	49.8 ± 7.88	18.22 ± 4.21	0.010*
Antibodies	Mean ± SD	Mean ± SD	
IgA (mg/dl)	292.2±78	183±93	0.031*
IgG	1851.2± 150.8	501.3 ± 75.71	0.022*
IgM	242.11 ± 92.5	37.4 ± 6.36	0.007*
HbF	16.87 ± 3.44	1.73 ± 0.09	0.0079*

Table 4: Effect of Splenectomy on immunological markers and HbF

Biological markers	Mean ± SD	P value	
Complement factors	**Non- Splenectomised**	**Splenectomised**	
C3 (mg/ml)	118.4	128.6 ± 11.7	0.057
C4 (g/ml)	47.3 ± 13.07	53.2 ± 12.8	0.076
Antibodies			
IgA (mg/dl)	200 ± 55.8	291.1 ± 76.4	
IgG	1722.6 ± 399.7	1868.3 ± 199.6	0.046*
IgM	204.8 ± 59.1	262.1 ± 50.9	2*
HbF	14.16 ± 3.88	18.22 ± 5.82	0.035*

The molecular study exhibited important differences ($p<0.05$) in the distribution of genotypes and alleles between sick and healthy persons, as shown in Table 5. The percentage of the mutant genotype CC in β-TM patients was double that in the control group, at 18% and 9%, respectively, while the heterogeneous genotype TC appeared in 60% of the patients and 27% of the healthy individuals. In addition to the above, the wild genotype TT appeared in 64% of the control and 22% of the patients, where it was present as a protective factor, as shown in table 5. The mutant allele C appeared as a genetic factor causing thalassemia in 48% of patients and 22% of healthy controls. In contrast, the wild allele T appeared as a protective factor in a high percentage (78%) in the control group compared to the patient group (52%).

The results of the existing training showed that the increase in blood transfusion of (splenectomy) in patients who carry the mutant genotype CC and who suffer from severe β-TM, where 50% of the splenectomized patients were carriers of the mutant genotype, while only 9% of non-splenectomized patients had that genotype. In contrast, 25% and 620.5% of patients with severe β-TM had mutant genotype CC and heterogeneous genotype TC, respectively. In addition, 18% and 73% of patients with moderate β-TM were carriers of the CC and TC genotypes, respectively. Similarly, most patients with mild β-TM had CC and TC values of 17% and 50%, respectively, as shown in Table (6). Moreover, mutation in the rs11886868 SNP of *BCL11A* was associated with a slight increase in HbF levels without a significant difference (P= 0.159) when compared with the level of HbF according to genotypes (15.21 g/dl, 15.6 g/dl, and 18.98 g/dl for TT, TC, and CC, respectively) table 6.

Table 5: Polymorphism of rs11886868 SNP of *BCL11A* gene in patients and controls

Polymorphism	Cases	Control	OR	P value	Z statistic	95%
Genotypes	N (%)	N (%)				
TT	10 (22)	29 (64)	0.158	0.0009	3.89	0.0621-
TC	27 (60)	13 (27)	4.125	0.0018	3.13	1.6938-
CC	8 (18)	4 (9)	2.2	0.049	1.219	0.6163-
Alleles						
T	47 (52)	70 (78)	312	0.028	3.528	1
C	43 (48)	20 (22)	0.3	0.0278	3.064	1.604-5848

*Significant Difference (P<0.05)

Table 6: Evaluation effect of rs11886868 SNP of *BCL11A* gene polymorphism on splenectomy and severity of β-TM

Parameters	Total No.	rs11886868 SNP	χ2	P value		
		TT	TC	CC		
Splenectomy		N (%)	N (%)	N (%)		
Splenectomised	10	3 (30)	2 (20)	5 (50)	13.76	0.003*
Non-	35	7 (20)	25 (71)	3 (9)	24..64	0.0019*
Severity of β-TM						
Sever	16	2 (12.5)	10 (62.5)	4(25)	2.09	0.046*
Intermediate	11	1 (9)	8 (73)	2 (18)	9.42	0.015*
Mild	18	6 (33)	9 (50)	3 (17)	11.33	0.0021*
HbF		15.21± 5.72	15.6 ± 4.92	18.98 ± 5.51	0.931	0.159

*Significant Difference (P<0.05)

DISCUSSION

β-TM institutes an essential genetic disease in Iraq, with the exacerbation of this problematic because of the instability, added to the financial and health difficulties that the country has suffered from it over the past few decades[22]. This study is the first in Iraq to deal with the role of the rs11886868 SNP of the BCL11A gene and some immunological indicators added to abnormal hemoglobin (HgF) in exacerbating the severity and development of thalassemia in children aged 5–14 years. Our study showed that β-TM is affected by the age and sex of the patients, as it appeared in children aged 9–14 years, especially females. The explanation for the increase in the rate of β-TM with age can be explained by the increase in blood diseases represented by the deformation of red blood cells and hemoglobin and disorders of the spleen and liver. We have not found a convincing explanation for the increase in the incidence of the disease among females [23].

Analysis of genome-wide association study data revealed a novel locus on chromosome 2, situated in the

BCL11A gene, that is linked to Hb F 24. The BCL11A gene encodes a zinc-finger protein that represses γ-globin gene expression. Hence, it hinders γ-globin chain synthesis and helps exchange Hb F for Hb A[25]. However, a single nucleotide polymorphism (SNP), rs11886868, in BCL11A has been linked to an increase in fetal hemoglobin synthesis. It has been demonstrated that the BCL11A gene's minor allele "C" of SNP rs11886868 is a main modifier of HbF ranks and an effector agent [26]. The study by Dadheecha et al. also found a significant correlation between the milder disease phenotype in β-thalassemia and the CC genotype and C allele [27]. The study population had a 40 percent frequency of the CC variant genotype. Nonetheless, in an Iranian population, the CC genotype was found to be prevalent in 28.8% of the population. Galanello et al.[28]. have recorded the occurrence of the CC genotype to be 61.2% in Sardinians. Moreover, the study by Uda et al.[29]. of the BCLL11A rs11886868 polymorphism revealed that the C allele was associated with a high Hb F percentage and was considerably more recurrent in patients with thalassemia intermedia. In contrast, the C allele was more common among Indonesian, Iranian, and Indian patients with thalassemia [30]. Chaouch et al. considered the C allele as the normal allele and the T allele as the mutant allele and observed that most patients with sickle cell anemia presented with HbF; more than 15% had CC genotype and ameliorated phenotype. Ethnic differences may account for the variation in the frequency of variant genotypes [31].

In adult erythroid precursors, the T/C variant (rs11886868) of the BCL11A gene results in downregulated BCL11A gene expression, which induces HbF and lessens the severity of β-thalassemia, according to previous research[32]. Experimental evidence of BCL11A's function as a regulator of γ-globin gene silencing has also been provided through the augmented production of HbF in developing adult erythroblasts following small hairpin RNA (sh-RNA) [33]. Owing to the physical interactions and co-expression of BCL11A and SOX6, BCL11A-mediated silencing is coordinated with the high-mobility group transcription factor SOX6. Data from functional and genetic studies indicate that BCL11A plays a crucial role in reactivating HbF in adult erythroblasts and in silencing γ-globin genes through progressive switching [34].

The immunological results showed that the serum concentrations of C3, C4, IgA, IgG, and IgM were elevated in β-TM patients compared with control. Thalassaemia patients are prone to numerous bacterial and viral contagions. Recurrent infections likewise motivate the immune system and might consequence in augmented immunoglobulin ranks[35]. Serum immunoglobulin levels rise as a result of chronic hepatitis, which is largely caused by HCV infection through blood transfusions. There are studies that show normal immunoglobulin levels in patients with β-thalassemia major, while other studies show elevated IgG and/or IgM levels as well as elevated IgA levels alone. Following splenectomy, thalassemia patients have shown a marked decline in serum IgM levels with no alteration in serum IgG or IgA ranks. However, splenectomized patients with β-thalassemia intermedia have been shown to have elevated serum IgG and IgA levels without a alteration in serum IgM levels. According to certain research, splenectomy has no effect on the normal serum Ig ranks in β-thalassemia patients [36].

Our Results showed increase serum level of IgA, IgM, and IgG among β-TM, especially Splenectomised βTM patients and this finding agrees with another study in Babylon City, according to a study by [37], splenectomy raises serum ranks of IgG and IgA (P value < 0.05) but has no effect on IgM levels. In contrast, Iraq demonstrated a highly significant increase in serum levels of IgA, IgM, and IgG among splenectomized patients [37]. It is thought that removing the spleen could put pressure on other secondary lymphoid organs to make up for the production of the main immunoglobulin classes, even though the spleen is one of the main lymphoid organs for eliminating blood infections. In dissimilarity to some additional trainings in the literature [38], study found that immunoglobulin levels increased while complement levels decreased [39]. Rather than being a primary issue, the detected immune illness primarily characterizes a secondary immune system fault. It appears that patients with β-thalassemia main have a fairly normal antibody reaction to bacterial and viral contagions with usual ranks of complement features, and these alterations cannot adequately explain the patients' increased susceptibility to infections. Increases in immunoglobulin levels appear to be followed by changes in complement factors, but it is not impossible that inadequate complement factor synthesis could be the cause [40].

CONCLUSION

This study showed there are differences in the distribution of rs11886868 SNP / *BCL11A* polymorphism, Antibodies, complement factors and HbF between β-TM patients and controls therefore; screening of *BCl11A* gene variants and serum concentration of immunoglobulins and complement factors in additional to HbF will transform the diagnosis, treatment, and prevention of β-TM in children and may eventually aid in predicting its severity.

Acknowledgments
The author would like to gratefully acknowledge the anonymous referees for the constructive and useful comments that they have provided.

Consent for Publication
Not applicable (no individual personal data included).
Funding: No external funds were received (private funding).
Conflict of Interest The authors affirm that their interests are not conflicting.
Availability of Data and Material
Data Access Statement: Research data supporting this publication are available at located

REFERENCES

1. Kattamis A, Kwiatkowski JL, Aydinok Y. Thalassaemia. The lancet. 2022;399(10343):2310-24.
2. Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of β-thalassemia. European journal of haematology. 2020;105(6):692-703.
3. Reading NS, Shooter C, Song J, Miller R, Agarwal A, Lanikova L, et al. Loss of Major DNase I Hypersensitive Sites in Duplicated β-globin Gene Cluster Incompletely Silences HBB Gene Expression. Human mutation. 2016;37(11):1153-6.
4. d'Arqom A. Nucleic acid therapy for β-thalassemia. Biologics: Targets and Therapy. 2020:95-105.
5. Farashi S, Harteveld CL. Molecular basis of α-thalassemia. Blood Cells, Molecules, and Diseases. 2018;70:43-53.
6. Liu N, Hargreaves VV, Zhu Q, Kurland JV, Hong J, Kim W, et al. Direct promoter repression by BCL11A controls the fetal to adult hemoglobin switch. Cell. 2018;173(2):430-42. e17.
7. Paikari A, Sheehan VA. Fetal haemoglobin induction in sickle cell disease. British journal of haematology. 2018;180(2):189-200.
8. Fard AD, Hosseini SA, Shahjahani M, Salari F, Jaseb K. Evaluation of novel fetal hemoglobin inducer drugs in treatment of β-hemoglobinopathy disorders. International journal of hematology-oncology and stem cell research. 2013;7(3):47.
9. Lai Z-S, Yeh T-K, Chou Y-C, Hsu T, Lu C-T, Kung F-C, et al. Potent and orally active purine-based fetal hemoglobin inducers for treating β-thalassemia and sickle cell disease. European Journal of Medicinal Chemistry. 2021;209:112938.
10. Soni S. Gene therapies for transfusion-dependent β-thalassemia. Indian Pediatrics. 2021;58(7):667-74.
11. Al-Allawi N, Al Allawi S, Jalal SD. Genetic epidemiology of hemoglobinopathies among Iraqi Kurds. Journal of community genetics. 2021;12(1):5-14.
12. Al-Harmooshee MBH, Al-Taei OMS. Germline Mutation of RAD51 Single Nucleotide Polymorphisms as Susceptibility Factor for Breast and Ovarian Cancer. Systematic Reviews in Pharmacy. 2020;11(10).
13. Mangia A, Bellini D, Cillo U, Laghi A, Pelle G, Valori VM, et al. Hepatocellular carcinoma in adult thalassemia patients: an expert opinion based on current evidence. BMC gastroenterology. 2020;20:1-14.
14. Danjou F, Anni F, Galanello R. Beta-thalassemia: from genotype to phenotype. haematologica. 2011;96(11):1573.
15. Tourkantoni N, Athanassiou-Metaxa M, Zafiriou D, Tzimouli V, Economou M, Taparkou A, et al. Immune and neural status of thalassemic patients receiving deferiprone or combined deferiprone and deferoxamine chelation treatment. Hemoglobin. 2008;32(1-2):35-40.
16. Politou M, Komninaka V, Valsami S, Kapsimali V, Pouliakis A, Koutsouri T, et al. The effect of transfusion on immune responses in thalassemia. Blood Cells, Molecules, and Diseases. 2020;83:102425.
17. Ahluwalia J, Datta U, Marwaha R, Sehgal S. Immune functions in splenectomized thalassaemic children. The Indian Journal of Pediatrics. 2000;67:871-6.
18. A, Jalali S, Amin R. Evaluation of the Serum Levels Immunoglobulin and Complement Factors in β- thalassemia Major Patients in Southern Iran. IJI, 2005; 2(4): p220-225.Al
19. Al-Taei OMS, editor Genetic Association between tumor necrosis factor (TNF-alpha and TNF-beta) gene polymorphisms and inflammatory bowel disease. Journal of Physics: Conference Series; 2020: IOP Publishing.
20. Sripichai O, Makarasara W, Munkongdee T, Kumkhaek C, Nuchprayoon I, Chuansumrit A, et al. A scoring system for the classification of β-thalassemia/Hb E disease severity. American journal of hematology. 2008;83(6):482-4.
21. Ghosh K, Colah R, Manglani M, Choudhry VP, Verma I, Madan N, et al. Guidelines for screening, diagnosis and management of hemoglobinopathies. Indian journal of human genetics. 2014;20(2):101.
22. AL-Damerchi AT, Kazaal MA. Evaluation of A Disintegrin and Metalloprotein33 Gene Polymorphism in Bronchial Asthma. Al-Qadisiyah Medical Journal. 2015;11(19):1-9.
23. Cappellini MD, Motta I. New therapeutic targets in transfusion-dependent and-independent thalassemia. Hematology 2014, the American Society of Hematology Education Program Book. 2017;2017(1):278-83.

24. Al-Allawi NA, Puehringer H, Raheem RA, Oberkanins C. Genetic modifiers in β-thalassemia intermedia: a study on 102 Iraqi Arab patients. Genetic testing and molecular biomarkers. 2015;19(5):242-7.
25. AbdElhameed AH, Mansour MM, Soliman MA, Helwa MA, El-Edel RH, Ragab SM. Genetic polymorphism of BCL11A (rs11886868) in Egyptian patients with β-thalassemia. Menoufia Medical Journal. 2021;34(1):305-9.
26. Al-Kenani HQM, Shaheed OM. Evaluation of some immunological markers in co-infection of COVID-19 with thrush candidiasis. Revista da Associação Médica Brasileira. 2024;70(4):e20230845.
27. Rujito L, Basalamah M, Siswandari W, Setyono J, Wulandari G, Mulatsih S, et al. Modifying effect of XmnI, BCL11A, and HBS1L-MYB on clinical appearances: A study on β-thalassemia and hemoglobin E/β-thalassemia patients in Indonesia. Hematology/oncology and stem cell therapy. 2016;9(2):55-63.
28. Nguyen TKT, Joly P, Bardel C, Moulsma M, Bonello-Palot N, Francina A. The XmnI Gγ polymorphism influences hemoglobin F synthesis contrary to BCL11A and HBS1L-MYB SNPs in a cohort of 57 β-thalassemia intermedia patients. Blood Cells, Molecules, and Diseases. 2010;45(2):124-7.
29. Dadheech S, Madhulatha D, Jain S, Joseph J, Jyothy A, Munshi A. Association of BCL11A genetic variant (rs11886868) with severity in β-thalassaemia major & sickle cell anaemia. Indian Journal of Medical Research. 2016;143(4):449-54.
30. Galanello R, Sanna S, Perseu L, Sollaino MC, Satta S, Lai ME, et al. Amelioration of Sardinian β0 thalassemia by genetic modifiers. Blood, The Journal of the American Society of Hematology. 2009;114(18):3935-7.
31. Uda M, Galanello R, Sanna S, Lettre G, Sankaran VG, Chen W, et al. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of β-thalassemia. Proceedings of the National Academy of Sciences. 2008;105(5):1620-5.
32. Neishabury M, Zamani F, Keyhani E, Azarkeivan A, Abedini SS, Eslami MS, et al. The influence of the BCL11A polymorphism on the phenotype of patients with beta thalassemia could be affected by the beta globin locus control region and/or the Xmn1-HBG2 genotypic background. Blood Cells, Molecules, and Diseases. 2013;51(2):80-4.
33. Chaouch L, Moumni I, Ouragini H, Darragi I, Kalai M, Chaouachi D, et al. rs11886868 and rs4671393 of BCL11A associated with HbF level variation and modulate clinical events among sickle cell anemia patients. Hematology. 2016;21(7):425-9.
34. Wilber A, Nienhuis AW, Persons DA. Transcriptional regulation of fetal to adult hemoglobin switching: new therapeutic opportunities. Blood, The Journal of the American Society of Hematology. 2011;117(15):3945-53.
35. Bazi A, Shahramian I, Yaghoobi H, Naderi M, Azizi H. The role of immune system in thalassemia major: a narrative review. Journal of Pediatrics Review. 2018;6(2):29-36.
36. Kiani-Amin M, Daneshi M, Ayazi P, Mohammadian S, Rezaei N. Serum immunoglobulin levels in splenectomized and non-splenectomized patients with major Beta-thalassemia. Iranian journal of pediatrics. 2011;21(1):95.
37. Ahmad HA, Abdullah TA. Estimation of Immunoglobulin and Complement proteins among Beta Thalassemia Major in Koya. Journal of Polytechnic.2015; 5(2):68-78
38. Aleem A, Shakoor Z, Alsaleh K, Algahtani F, Iqbal Z, Al-Momen A. Immunological evaluation of β-thalassemia major patients receiving oral iron chelator deferasirox. J Coll Physicians Surg Pak. 2014;24(7):467-71.
39. Piomelli S, Karpatkin M, Arzanian M, Zamani M, Becker M, Geneiser N, et al. Hypertransfusion regimen in patients with Cooley's anemia. Annals of the New York Academy of Sciences. 1974;232(1):186-92.
40. Propper R. Transfusion management of thalassemia. Methods in hematology the thalassemia, 3rd ed Churchil Livingston. 1983:145-61.