Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

The Impact of the Rs3806596; T>C Genetic Polymorphism of UDP Enzyme on the Response to Deferasirox Drug Among Iraqi Thalassemia Patients

Husam Saleh Hasan[1*], Ahmed H. Ismael[2], Hasan Moosa Abed[3]

[1]Department of Pharmacology and Toxicology, College of Pharmacy, University of Karbala, Karbala, Iraq
[2]Department of Clinical Pharmacy, College of Pharmacy, University of Kerbala, Karbala, Iraq
[3]Hereditary Blood Diseases Center, Kerbala, Iraq

[*]**Corresponding author**
Husam Saleh Hasan: husam.s@s.uokerbala.edu.iq

Received: 15/12/2024
Accepted: 20/01/2025
Published: 30/06/2025

Keywords: Thalassemia, rs3806596; T>C, UGT1A3, deferasirox, chelation therapy.

DOI:
10.62472/kjps.v16.i26.137-146

Abstract

Background: thalassemia is an autosomal recessive hereditary condition, Patients with beta thalassemia need ongoing blood transfusions and iron chelating treatment due to the body's inability to excrete iron. Clinical observations of transfusion dependent thalassemia (TDT) patients in the local community show that many of them continue to have elevated blood ferritin levels even after receiving treatment with the iron chelator Deferasirox (DFX). One of the primary UGT1 enzyme families that metabolizes DFX is the UDP-glucuronosyltransferase 1A3 (UGT1A3) gene. This study examines the impact of the UGT1A3 gene's rs3806596; T>C single nucleotide polymorphism (SNP) on Iraqi TDT patients' clinical response to DFX chelation treatment.

Methodology: Cross-sectional study included 96 TDT patients of Iraqi male and female.They were administered with 30–40 mg/kg of oral DFX daily for at least 3 months, and they ranged in age from 8 to 39. Serum erythroferrone, liver and kidney function tests, and serum ferritin were evaluated. The method of allele-specific polymerase chain reaction was used to identify the rs3806596; T>C SNP.

Results: The genotype distribution of rs3806596; T>C SNP among Iraqi population was 36.5%, 18.5% and 44.8%, for TT, TC and CC respectively. The frequency of the wild (T) allele is 0.46, compared to 0.54 for the mutant (C) allele. The mutant group (CC) of rs3806596; T>C SNP showed no significant in the three different groups.

Conclusion: There is no significant association between rs3806596; T>C SNP of the UGT1A3 gene and the therapeutic response to DFX in our samples of Iraqi population for TDT patients.

تأثير التعدد الشكلي الجيني للطفرة Rs3806596; T>C لإنزيم UDP على استجابته لدواء ديفيراسيروكس بين مرضى الثلاسيميا العراقيين

حسام صالح حسن، احمد حقي اسماعيل، حسن موسى عبد

الخلاصة

الثلاسيميا هي حالة وراثية متنحية تؤثر على إنتاج الهيموجلوبين، ويحتاج مرضى الثلاسيميا بيتا إلى عمليات نقل دم مستمرة، كما أن العلاج بالاستخلاب بالحديد ضروري بسبب عدم قدرة الجسم على طرح الحديد بشكل طبيعي. ومع ذلك، تُظهر العلامات السريرية لمرضى الثلاسيميا المعتمدين على نقل الدم (TDT) في المجتمع المحلي أن العديد منهم يستمرون في الحصول على مستويات مرتفعة من الفيريتين في الدم حتى بعد تلقي العلاج باستخدام عقار ديفيراسيروكس (DFX). إحدى عائلات إنزيم UGT1 الأساسية التي تستقلب عقار ديفيراسيروكس هي جين UDP-glucuronosyltransferase 1A3 (UGT1A3). تتبين هذه الدراسة تأثير تعدد أشكال النوكليوتيدات المفردة (SNP) لجين UGT1A3 على الاستجابة السريرية لمرضى الثلاسيميا العراقيين المعتمدين على نقل الدم لعلاج الاستخلاب بعقار ديفيراسيروكس.

العينات وطرق العمل

شملت هذه الدراسة المقطعية ستة وتسعين مريضًا مصابين بالثلاسيميا المعتمدين على نقل الدم (TDT) من الذكور والإناث العراقيين، تم إعطاؤهم جرعة 30–40 مجم / كجم من DFX عن طريق الفم يوميًا لمدة ثلاثة أشهر على الأقل، وتراوحت أعمارهم بين 8 إلى 39 عامًا. تم تقييم مصل إريثروفيرون ومصل الفيريتين واجراء اختبارات لوظائف الكبد والكلى. تم استخدام طريقة تفاعلات الكوثرة المتسلسلة النوعية للألليل لتحديد تأثير التعدد الشكلي الجيني للطفرة rs3806596; T>C.

النتائج

اثبت ان توزيع النمط الجيني لـrs3806596; T>C بين السكان العراقيين هو 36.5٪ و 18.5٪ و 44.8٪، لـ TT و TC و CC على التوالي. حيث كان معدل الأليل السائد (T)هو (0.46)، مقارنة بـ (0.54) للأليل المتنحي (T). لم تظهر المجموعة المتنحية (CC) من rs3806596؛ T>C SNP أي تأثير أو أهمية بين المجموعات الثلاث المختلفة.

الاستنتاج

لا يوجد تأثير واضح بين rs3806596؛ T>C SNP لجين UGT1A3 والاستجابة العلاجية لـ DFX في عيناتنا من السكان العراقيين لمرضى TDT.

1. Introduction

Beta-thalassemia is an autosomal recessive hereditary condition that affects the β-chain of hemoglobin production. In the Eastern Mediterranean area, including Iraq, the disease is prevalent (Yahya, 1996, Hamamy and Al-Allawi, 2013). The β-thalassemia mutations cause a range of clinical symptoms, starts from thalassemia minor in individuals who are heterozygous for it to β-thalassemia major (β-TM) in those who are homozygous or compound heterozygous , β-TM phenotype is aserious condition linked to several problems with a lifelong need for blood transfusions, stunted growth, diabetes mellitus, liver illness, hypothyroidism, hypoparathyroidism and hypogonadism are among the most common consequences (Galanello and Origa, 2010). With a significantly higher quality of life and fewer problems in developed compared to developing nations, the frequency of these issues vary somewhat throughout β-TM cohorts and are related to the effectiveness of care and follow-up given to these patients (Mikael and Al-Allawi, 2018). Patients should usually start iron chelation treatment when their ferritin levels above 1000 ng/ml or after ten to twenty times blood transfusions (Cappellini et al., 2013). Excessive serum iron can increase the morbidity and death if it is not treated appropriately (Tanaka, 2014). Chelation therapy with parenteral deferoxamine has been the standard treatment for iron overload, but patient compliance is often low due to the discomfort and demands of its administration regimen. Therefore, significant emphasis has been placed on creating oral chelating agents. Deferiprone has been available in Asia for nearly twenty years and has shown promising results for cardiac iron removal and long-term effectiveness. However, its serious side effects, such as agranulocytosis and neutropenia, have hindered its widespread use in clinical settings. Deferasirox (DFX) is a new oral chelator that effectively eliminates iron from the heart and liver over the course of a 24-hour dosage period, according to preclinical and clinical studies. The best way to treat patients with severe thalassemia is the accessibility of oral iron chelators, which remain significant obstacles in many Asian nations (Viprakasit et al., 2009). In more than 70 country worldwide, including the US in 2005 and the EU in 2006, DFX has been approved as a first-line therapy for thalassemia major (Cappellini et al., 2013). DFX is a tridentate ligand that is achiral and exhibits a strong affinity and selectivity for ferric iron (Fe3+) (Nick, 2007). Only over 8% of DFX and its metabolites are removed by the kidneys; the majority, roughly 84%, is removed in the feces. Uridine glucuronosyltransferase (UGT) is the primary enzyme responsible for the drug's substantial metabolism by glucuronidation (Allegra et al., 2017). DFX at dosages of 10 to 40 mg/kg was found to reduce liver iron, lower serum ferritin and increase iron excretion in thalassemia patients (Cappellini et al., 2013). Prolonged DFX treatment may have serious side effects, such as bone marrow suppression, gastrointestinal bleeding, liver and kidney failure (Cusato et al., 2015). The essential metabolic route for DFX is glutaronidation, which is primarily carried out by UDP glucuronosyltransferase family 1 member A1 (UGT1A1) and, to a lesser degree, by UDP glucuronosyltransferase family 1 member A3 (UGT1A3)(Agent, 2021). Through a process known as glutaronidation, phase 2 conjugation routes enhance the clearance of a variety of substances by increasing their solubility in water (Nagar and Blanchard, 2006).The UGT1A3 gene produces a member of the UDP-glucuronosyltransferase (UGT) family, which is involved in the glucuronidation process, which is a vital part of the metabolism of many endogenous and exogenous substances. The detoxification and removal of medications, hormones, and other chemicals from the body depend heavily on this enzyme activity and expression. Individuals' susceptibility to specific diseases and how they react to drugs may be impacted by variations in the UGT1A3 gene (Mullapudi et al., 2021), which certainly affect the drug serum concentration resulting in variation in drug response. Although the liver is where UGT1A3 is mostly expressed, it is also present in the bile ducts, the stomach

and the intestines (Sabolovic et al., 2000). The single UGT1 gene on chromosome 2q37 encodes UGT1A3. All eight additional functional UGT isoforms expressed from the UGT1A locus share four of the five exons that make up the UGT1A3 mRNA, which encodes the C-terminal half of the molecule(Caillier et al., 2007).

The rs3806596; T>C single nucleotide polymorphism (SNP) lies inside the UGT1A3 gene's main promoter region, specifically located. A previous research has shown that polymorphisms in the promoter region of UGT1A3 are linked to differences in drug responses and the risk of various diseases (Mullapudi et al., 2021). This study aimed to investigate the impact of the rs3806596; T>C SNP in the UGT1A3 gene on the therapeutic response to DFX.

2. Materials and Methods

2.1. Study Subjects

The Scientific and Ethical Committee of Karbala University College of Pharmacy examined and approved this cross-sectional study. (Reference number: 2023HU7).

Between November 2023 and April 2024, 96 individuals with iron excess beta-thalassemia major (specifically transfusion-dependent thalassemia, or TDT) were selected from a total of 650 patients at Karbala Teaching Hospital for Children, a thalassemia specialty center. The patients were between the ages of 8 and 39. Prior to being recruited for the study, each participant provided written informed consent that included a detailed explanation of the study's goals. The participants were also given a specially designed questionnaire to complete. The subjects had been receiving DFX chelation medication as monotherapy for a minimum of three months. The highest dose of DFX that is advised by the Thalassemia International Federation is 30–40 mg/kg/day; this is the same dose that was chosen for this study. Alcohol users, patients with sickle cell anemia, patients with liver illness and patients with thalassemias other than beta-thalassemia were excluded from the study. Patients having any medical or surgical problems that may substantially impact a drug's absorption, metabolism, or excretion were also not allowed to participate in this study.

Using a disposable syringe, five milliliters of blood were extracted from each participant's vein(Miran et al., 2024). A plain tube devoid of anticoagulants was filled with 2.5 milliliters of blood, which was allowed to coagulate for around half an hour. The serum required to assess the amounts of human erythroferrone (ERFE) and other biomarkers was extracted from the blood samples by centrifuging them at a speed of 4000 x g. Before being utilized, the sera samples were kept at -20 °C. The second portion of the blood sample (2.5 ml) was transferred into evacuated ethylenediaminetetraacetic acid (EDTA) tubes, for other hematological testing and DNA extraction.

2.2. Genetic Analysis

Following the manufacturer's instructions, the genomic DNA was extracted from the whole blood samples using the Geneaid DNA Extraction/Genomic DNA Purification Prep Mini Kit (Taiwan). An allele-specific polymerase chain reaction (PCR) method was used to find the rs3806596; T>C (SNP). The lyophilized primers were developed with the Primer-BLAST software and provided by Macrogen-Korea(Kadhim and Gaaib, 2024). Each primer was dissolved in a predetermined volume of nuclease-free water to create a stock solution with a concentration of 100 pmol/µL. The diluted (10 pmol/µL) working solutions were made by mixing 90 µL of nuclease-free water with 10 µL of each forward and reverse primer stock solution. The working solutions were stored at -20 °C until they were utilized. The primer sequences are as follows: The forward primer is 5′-ATCCTGGTGCGAAAAACGA-3′, the first reverse primer is 5′ CCTGCTACATTTGCTTTCTTCA-3′, and the second reverse primer is 5′-CCTGCTACATTTGCTTTCTTCG-3′. 12 µL of master mix (Promega, United States

of America), 2 µL (100 ng/µL) of the extracted DNA, 1 µL (10 pmol/µL) of the forward primer, 1 µL (10 pmol/µL) of the reverse primer, and 9 µL of nuclease-free water made up the 25 µL PCR reaction mixture. The following programming conditions were used for the PCR amplification: a 3-minute initial denaturation step at 95 °C, followed by 30 cycles of amplification that included 30 seconds of denaturation at 95 °C, 50 seconds of annealing at 64 °C, and 40 seconds of extension at 72 °C. The last extension phase was carried out at 72 °C for five minutes. Electrophoresis was performed on a 2% (w/v) agarose gel to confirm the amplification and identify the PCR products.

2.3. Biochemical Analysis

Red blood cell (RBC) and hemoglobin (Hb) levels in patients are precisely measured with the Swelab Alfa plus Basic Hematology Analyzer, which provides crucial information for evaluating blood health and identifying any medical disorders. A Sunlong Biotech kit was used to assess human ERFE levels using an enzyme-linked immunosorbent assay (ELISA) sandwich technique. Human Humareader HS and Human Combiwash from Germany were used to process the test. While the Mindray BS 240 system was used to perform the liver and kidney function tests, the Cobas e411 analyzer from Germany was used to detect the levels of serum ferritin (SF).

2.4. Statistical Analysis

Each participant received a unique identifying number, and a data sheet was used to record their responses. Multiple entries were made for verification in order to minimize mistakes. The Statistics Package for the Social Sciences, 22nd edition (SPSS Inc., Chicago, US), was used to analyze the study data.

The Chi-square value was obtained by using the goodness of fit test to determine if Hardy-Weinberg equilibrium applied to allele distribution. For two groups, the T-test was used to determine the means of the hematological and biochemical parameters; for three or more groups, a one-way ANOVA was used. A p-value of less than 0.05 was deemed to indicate a meaningful change. (AL-Safar et al., 2024)

3. Results

This study included ninty-six Iraqi patients with major β-thalassemia, 42 of whom were male and 54 of whom were female. The rs3806596; T>C SNP was found using allele-specific PCR. Whether T or C alleles were present affected the PCR amplification. The T column of the agarose gel showed a single 519 bp PCR band for the wild type (TT). Two PCR bands, each measuring 519 bp in size were seen in the T and C columns for the heterozygous mutant type (TC). Only the C column showed a single 519 bp PCR band for the homozygous mutant type (CC). Fig.1 illustrates the outcomes.

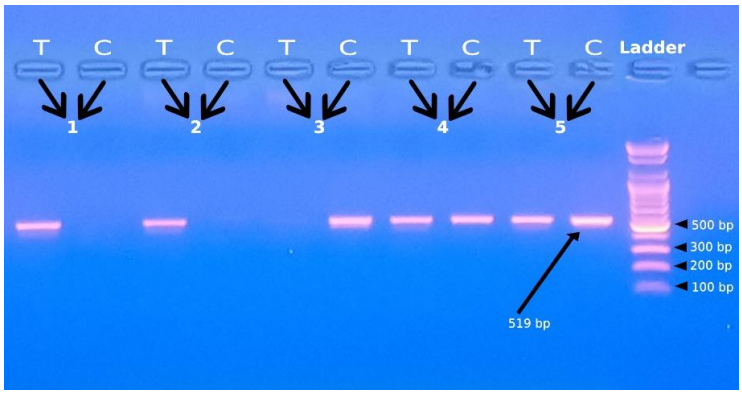

Figure1: Agarose Gel Electrophoresis Employing Allele-Specific PCR to Detect the Rs3806596; T>C SNP. Wild type (TT) is represented by lanes 1 and 2, heterozygous mutant type (TC) by lane 4 and 5 and homozygous mutant type (CC) by lane 3.

The sex distribution and other demographic properties of the study participants are demonstrated in Table1. According to the patient demographics, the participants' ages ranged from 8 to 39 years old, with around 67% of them being under 19 and just two of them being older than 30. About 82.3% of the participants were urban residency while only 17.7% were rural. Based on marital relativity or paternal consanguinity, the research groups were split into two sex distribution and other demographic characteristics. The rs3806596; T>C SNP genotype distribution revealed that the wild type (TT) allele is making up around 36.5% of individuals, whereas the mutant (CC) allele only makes up 44.8% of the research participants Table2. The patients were divided into three groups according to their genotype. Notably, the analysis reveals no significant differences were observed in the hematological and biochemical parameters Table3.

Table 1: Demographic Characteristics of the Study Subjects

Character		Frequency (%)
Sex	Male	42(43.4)
	Female	54(56.3)
Age	8-18 years old	64 (66.6)
	19-39 years old	32 (33.3)
Residency	Urban	79(82.3)
	Rural	17(17.7)
Marital relativity	Yes	85(88.5)
	No	11(11.5)
Blood group	A	32(33.3)
	B	22(22.9)
	AB	9(9.4)
	O	33(34.4)

Table2: The Genotype Distribution of rs3806596; T>C SNP Among the Study Subjects

Genotype	Frequency (%)	Chi squared	P value
TT	35(36.5)	37.19	0.0001
TC	18(18.8)		
CC	43(44.8)		
Allele	Frequency		
T	0.46		
C	0.54		

Table 3: The Association Between Rs3806596; T>C SNP And the Hematological and Biochemical Parameters

Parameter	TT (n=35) Mean ±SD	TC (n=18) Mean ±SD	CC (n=43) Mean ±SD	P value
Age (year)	17.11±1.11	17.05±1.53	15.02±1.09	0.34
BMI (kg/m^2)	18.12±0.63	18.84±0.69	18.31±0.50	0.76
Dose (mg/Kg/day)	34.02±0.53	33.72±0.86	33.76±0.61	0.93
SF (ng/ml)	3432.34±376.84	3060.88±365.47	3324.97±323.68	0.72
AST (U/L)	38.34±2.43	39.91±5.66	40.89±5.63	0.72
ALT (U/L)	34.78±4.53	32.13±6.01	39.81±6.77	0.53
S. creatinine (mg/Dl)	0.36±0.03	0.36±0.03	0.29±0.01	0.13
Blood urea (mg/dL)	26.73±1.33	26.56±1.72	25.76±1.51	0.87
TSB (mg/dL)	1.74±0.15	1.75±0.14	2.25±0.18	0.06
ERFE (ng/L)	39.42±2.82	53.00±8.16	43.72±4.58	0.18
RBC (Million/mm3)	3.06±0.09	2.98±0.15	2.88±0.07	0.35
HB (g/ dL)	8.10±0.18	8.20±0.24	7.90±0.14	0.51
Blood TF (Days)	21.97±2.78	18.27±0.89	19.02±0.83	0.37

4. Discussion

This study looked at how the rs3806596; T>C SNP affected the therapeutic response to the DFX medication in TDT patients from Iraq. People with thalassemic syndrome may get iron overload as a result of frequent blood transfusions. Since the body lacks the ability to remove excess iron, an iron-chelation therapy, such as a DFX medication, must be taken (Chaudhary and Pullarkat, 2013, Naderi et al., 2013). The symptoms of thalassemia range from basic chronic tiredness and arthropathy to cardiomyopathy, diabetes, and liver cirrhosis, and many individuals are unresponsive to DFX. Pharmacogenetic and demographic researches are crucial for determining the optimal therapeutic response and more precisely identifying potential causes of treatment failure.

TDT is positively impacted by consanguinity, especially in nations where marriages between cousins are widespread (64.6% of the Iraqi population) (Yahyaa et al., 2019). Consanguinity seems to have a significant role in the extent of the problem in Iraq; about 88.5% of the patients in this research were the children of first- and second-cousin marriages. Similar findings (88%) were observed by other previous studies (Al-Haj, 1992), (Adaay et al., 2011), whereas Awad reported a lower result (41.6%) in his study (Awad, 1999). A, B, AB, and O were the four groups into which the research participants were assigned based on the ABO blood group classification. The blood group O had the largest percentage among TDT patients (34.4%), followed by the A blood group (33.3%), while the blood group AB had the lowest rate (9.4%). These findings were consistent with earlier research on the Iraqi population (Marbut et al., 2018, Adaay et al., 2011). According to the current study, the patients' BMI is around 18 kg/m^2, and several previous studies show that over half of β-thalassemia patients are underweight (Salih and Al-Mosawy, 2013, Soliman et al., 2023, Hammod et al., 2018). Our findings are mostly explained by the fact that the majority of participants (66.6%) were under the age of 18 (Table 1). Thalassemia has historically been associated with a 30% to 60% risk of short height and developmental failure in children. However, the risk of short stature has considerably lowered with current adherence to modern transfusion and iron chelation regimens, as well as cautious treatment to prevent iron chelator overdose, potentially enhancing endocrine development in children with TDT (Farmakis et al., 2022). Based on their genotype, the research participants were split into three groups: TT, TC, and CC groups. This study showed that 36.5% of the patients carry the wild type (TT), 18.8% carry TC and 44.8% carry the CC genotype, this means that the prevalence of rs3806596; T>C SNP in the study subjects is 63.6%. The Thalassemia International Federation Guidelines 2021 for (TDT) state that DFX should be taken at a dose of 10–40 mg/kg per day (Babu and Panachiyil, 2022). Only patients who received 30–40 mg/kg/day of DFX as their daily dose were included in this study in order to identify the cases that were not responsive to chelation treatment, even at high doses, and to investigate any possible association with genetic polymorphism analysis. Hematological and biochemical indicators did not significantly differ between patient groups. Serum ferritin (SF) is a crucial indicator of excessive iron (Farmakis et al., 2022). It is a simple and reasonably priced test for assessing iron overload. The TDT Guidelines 2021 state that its levels are used to determine if DFX treatment is being received appropriately or insufficiently. They are connected with the total body iron burden (Chirnomas et al., 2009, Brittenham et al., 1993) .Since DFX is metabolized by the liver glucuronidation, mainly by UGT1A1,UGT1A3 as well as bilirubin (Waldmeier et al., 2010) genetic polymorphism of rs3806596; T>C SNP show no significant results or impact on gene expression in our study of Iraqi population as the following previous study (Cao et al., 2020), while in other study there was a significant result for this SNP in certain different population (Cusato et al., 2016). A glycoprotein hormone called erythroferrone functions as a "responsive erythroid" regulation element for erythropoietin (Saad et al., 2021).

Because of inefficient erythropoiesis, ERFE is elevated in thalassemia and plays a critical role in controlling the release of stored iron (Kautz et al., 2014b, Kautz and Nemeth, 2014). It also mediates the suppression of the iron-regulatory hormone hepcidin, which promotes iron intake and mobilization from storage, and is essential for the recovery of hemorrhage-induced anemia (Kautz et al., 2014a). No significant results has been proved for this study, this confirm that the rs3806596; T>C SNP has no impact on the UGT1A3 gene in DFX users. Nephrotoxicity from deferasirox may show up as a little increase in blood creatinine levels. However, there is currently no clearly defined threshold value for determining nephrotoxicity,(Cappellini et al., 2011, Cappellini et al., 2006, Cappellini et al., 2010). A reduction in glomerular filtration rate, either acute or chronic, is one of the nephrotoxic DFX side effects. Nephrotoxicity's molecular mechanisms are still poorly understood, despite the fact that it is frequently reversible and/or non-progressive (Díaz-García et al., 2014). In the present study serum creatinine showed normal values.

5. Conclusion

There is no significant association between rs3806596; T>C SNP in the UGT1A3 gene and the hematological and biochemical parameters in Iraqi thalassemia patients treated with deferasirox.

6. Conflicts of Interest

The authors declare no conflict of interests

References

ADAAY, M. H., AL-ANZY, M. M., AL-SAMARRAI, A.-M. H., AL-TIKRITI, K. A. & AL-SAMARRAI, F. A. 2011. Some Observations on the Occurrence of β-Thalassemia in Mosul. *Iraqi J. Med. Sci,* 9**,** 270-274.

AGENT, A. 2021. PRODUCT MONOGRAPH INCLUDING PATIENT MEDICATION INFORMATION.

AL-HAJ, F. 1992. *Haemoglobinopathies in Mosul.* MSc Thesis University of Mosul Iraq.

AL-SAFAR, S. R. M., AL-ZUBAIDY, H. F. & ALWAIDH, R. H. 2024. An Investigation of the Correlation Between the CNTNAP2 rs7794745 gene Polymorphism and Autism in Children from the Middle Euphrates Area of Iraq. *Karbala Journal of Pharmaceutical Sciences,* 15**,** 1-10.

ALLEGRA, S., DE FRANCIA, S., CUSATO, J., ARDUINO, A., MASSANO, D., LONGO, F., PIGA, A. & D'AVOLIO, A. 2017. Deferasirox pharmacogenetic influence on pharmacokinetic, efficacy and toxicity in a cohort of pediatric patients. *Pharmacogenomics,* 18**,** 539-554.

AWAD, M. 1999. *Homozygous β-thalassemia in Mosul.* PhD thesis, Mosul Univ, Iraq.

BABU, T. & PANACHIYIL, G. M. 2022. An Evidence-Based Approach to Treatment with Iron Chelators in Transfusion-Dependent Thalassemia Patients: Present Trends and Future Scenario. *Frontiers in Clinical Drug Research-Hematology,* 5**,** 50.

BRITTENHAM, G. M., COHEN, A. R., MCLAREN, C. E., MARTIN, M. B., GRIFFITH, P. M., NIENHUIS, A. W., YOUNG, N. S., ALLEN, C. J., FARRELL, D. E. & HARRIS, J. W. 1993. Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. *American journal of hematology,* 42**,** 81-85.

CAILLIER, B., LÉPINE, J., TOJCIC, J., MÉNARD, V., PERUSSE, L., BÉLANGER, A., BARBIER, O. & GUILLEMETTE, C. 2007. A pharmacogenomics study of the human estrogen glucuronosyltransferase UGT1A3. *Pharmacogenetics and genomics,* 17**,** 481-495.

CAO, K., REN, G., LU, C., WANG, Y., TAN, Y., ZHOU, J., ZHANG, Y., LU, Y., LI, N. & CHEN, X. 2020. ABCC2 c.-24 C> T single-nucleotide polymorphism was associated with the pharmacokinetic variability of deferasirox in Chinese subjects. *European Journal of Clinical Pharmacology,* 76**,** 51-59.

CAPPELLINI, M.-D., COHEN, A., ELEFTHERIOU, A., PIGA, A., PORTER, J. & TAHER, A. 2013. Guidelines for the clinical management of thalassaemia.

CAPPELLINI, M. D., BEJAOUI, M., AGAOGLU, L., CANATAN, D., CAPRA, M., COHEN, A., DRELICHMAN, G., ECONOMOU, M., FATTOUM, S. & KATTAMIS, A. 2011. Iron chelation with deferasirox in adult and pediatric patients with thalassemia major: efficacy and safety during 5 years' follow-up. *Blood, The Journal of the American Society of Hematology,* 118**,** 884-893.

CAPPELLINI, M. D., COHEN, A., PIGA, A., BEJAOUI, M., PERROTTA, S., AGAOGLU, L., AYDINOK, Y., KATTAMIS, A., KILINC, Y. & PORTER, J. 2006. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with β-thalassemia. *Blood,* 107**,** 3455-3462.

CAPPELLINI, M. D., PORTER, J., EL-BESHLAWY, A., LI, C.-K., SEYMOUR, J. F., ELALFY, M., GATTERMANN, N., GIRAUDIER, S., LEE, J.-W. & CHAN, L. L. 2010. Tailoring iron chelation by iron intake and serum ferritin: the prospective EPIC study of deferasirox in 1744 patients with transfusion-dependent anemias. *haematologica,* 95**,** 557.

CHAUDHARY, P. & PULLARKAT, V. 2013. Deferasirox: appraisal of safety and efficacy in long-term therapy. *Journal of blood medicine***,** 101-110.

CHIRNOMAS, D., SMITH, A. L., BRAUNSTEIN, J., FINKELSTEIN, Y., PEREIRA, L., BERGMANN, A. K., GRANT, F. D., PALEY, C., SHANNON, M. & NEUFELD, E. J. 2009. Deferasirox pharmacokinetics in patients with adequate versus inadequate response. *Blood, The Journal of the American Society of Hematology,* 114**,** 4009-4013.

CUSATO, J., ALLEGRA, S., DE FRANCIA, S., MASSANO, D., PIGA, A. & D'AVOLIO, A. 2016. Role of pharmacogenetics on deferasirox AUC and efficacy. *Pharmacogenomics,* 17**,** 571-582.

CUSATO, J., ALLEGRA, S., MASSANO, D., DE FRANCIA, S., PIGA, A. & D'AVOLIO, A. 2015. Influence of single-nucleotide polymorphisms on deferasirox Ctrough levels and effectiveness. *The Pharmacogenomics Journal,* 15**,** 263-271.

DÍAZ-GARCÍA, J. D., GALLEGOS-VILLALOBOS, A., GONZALEZ-ESPINOZA, L., SANCHEZ-NINO, M. D., VILLARRUBIA, J. & ORTIZ, A. 2014. Deferasirox nephrotoxicity—the knowns and unknowns. *Nature reviews nephrology,* 10**,** 574-586.

FARMAKIS, D., PORTER, J., TAHER, A., CAPPELLINI, M. D., ANGASTINIOTIS, M. & ELEFTHERIOU, A. 2022. 2021 Thalassaemia international federation guidelines for the management of transfusion-dependent thalassemia. *Hemasphere,* 6**,** e732.

GALANELLO, R. & ORIGA, R. 2010. Beta-thalassemia. *Orphanet journal of rare diseases,* 5**,** 1-15.

HAMAMY, H. A. & AL-ALLAWI, N. A. 2013. Epidemiological profile of common haemoglobinopathies in Arab countries. *Journal of community genetics,* 4**,** 147-167.

HAMMOD, H., MOKIF, T. A. & AL-HARBI, H. J. 2018. The correlation between thalassemia with body mass index and blood groups in children and adult patient in the province of Babylon, Iraq. *Asian Journal of Pharmaceutical and Clinical Research,* 11**,** 509-512.

KADHIM, Z. A.-H. & GAAIB, J. N. 2024. Association of FTO Gene Variants with Some Biochemical Markers of Type 2 Diabetes Mellitus Patients in Iraqi Population. *Karbala Journal of Pharmaceutical Sciences,* 15**,** 106-123.

KAUTZ, L., JUNG, G., NEMETH, E. & GANZ, T. 2014a. Erythroferrone contributes to recovery from anemia of inflammation. *Blood, The Journal of the American Society of Hematology,* 124**,** 2569-2574.

KAUTZ, L., JUNG, G., VALORE, E. V., RIVELLA, S., NEMETH, E. & GANZ, T. 2014b. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nature genetics,* 46**,** 678-684.

KAUTZ, L. & NEMETH, E. 2014. Molecular liaisons between erythropoiesis and iron metabolism. *Blood, The Journal of the American Society of Hematology,* 124**,** 479-482.

MARBUT, S. M. M., HAMDI, M. A., JUMAA, A. M. & SALMAN, B. A. 2018. Distribution of ABO blood groups in beta thalassemia patients dependent on blood transfusion In Bagdad city. *Journal of Madenat Alelem University College,* 10**,** 1-11.

MIKAEL, N. A. & AL-ALLAWI, N. A. 2018. Factors affecting quality of life in children and adolescents with thalassemia in Iraqi Kurdistan. *Saudi medical journal,* 39**,** 799.

MIRAN, N. Z., JABBAR, S., AL-KAREEM, Z. A. & ALZAJAJI, Q. B. 2024. Studying the Role of L-Thyroxine in Long-Term Management of Hypothyroidism: A Comparative Analysis with Healthy Control. *Karbala Journal of Pharmaceutical Sciences,* 15**,** 35-56.

MULLAPUDI, T. R., RAVI, P. R. & THIPPARAPU, G. 2021. UGT1A1 and UGT1A3 activity and inhibition in human liver and intestinal microsomes and a recombinant UGT system under similar assay conditions using selective substrates and inhibitors. *Xenobiotica,* 51**,** 1236-1246.

NADERI, M., SADEGHI-BOJD, S., VALESHABAD, A. K., JAHANTIGH, A., ALIZADEH, S., DORGALALEH, A., TABIBIAN, S. & BAMEDI, T. 2013. A prospective study of tubular dysfunction in pediatric patients with beta thalassemia major receiving deferasirox. *Pediatric hematology and oncology,* 30**,** 748-754.

NAGAR, S. & BLANCHARD, R. L. 2006. Pharmacogenetics of uridine diphosphoglucuronosyltransferase (UGT) 1A family members and its role in patient response to irinotecan. *Drug metabolism reviews,* 38**,** 393-409.

NICK, H. Deferasirox (Exjade®, ICL670) preclinical overview. Seminars in hematology, 2007. Elsevier, S12-S15.

SAAD, H., BAHGAT, S. M., EL DEWY, D. M. & GALAL, R. K. 2021. Erythroferrone level in β-thalassemia patients. *Journal of Recent Advances in Medicine,* 2**,** 1-9.

SABOLOVIC, N., MAGDALOU, J., NETTER, P. & ABID, A. 2000. Nonsteroidal anti-inflammatory drugs and phenols glucuronidation in Caco-2 cells: identification of the UDP-glucuronosyltransferases UGT1A6, 1A3 and 2B7. *Life sciences,* 67**,** 185-196.

SALIH, K. & AL-MOSAWY, W. 2013. Evaluation some consequences of thalassemia major in splenectomized and non-splenectomized Iraqi patients. *Int J Pharm Pharm Sci,* 5**,** 385-358.

SOLIMAN, A., YASSIN, M., ALYAFEI, F., ALAARAJ, N., HAMED, N., OSMAN, S. & SOLIMAN, N. 2023. Nutritional studies in patients with β-thalassemia major: A short review. *Acta Bio Medica: Atenei Parmensis,* 94.

TANAKA, C. 2014. Clinical pharmacology of deferasirox. *Clinical pharmacokinetics,* 53**,** 679-694.

VIPRAKASIT, V., LEE-LEE, C., CHONG, Q. T., LIN, K.-H. & KHUHAPINANT, A. 2009. Iron chelation therapy in the management of thalassemia: the Asian perspectives. *International journal of hematology,* 90**,** 435-445.

WALDMEIER, F., BRUIN, G. J., GLAENZEL, U., HAZELL, K., SECHAUD, R., WARRINGTON, S. & PORTER, J. B. 2010. Pharmacokinetics, metabolism, and disposition of deferasirox in β-thalassemic patients with transfusion-dependent iron overload who are at pharmacokinetic steady state. *Drug Metabolism and Disposition,* 38**,** 808-816.

YAHYA, H. I. 1996. Thalassaemia genes in Baghdad, Iraq. *EMHJ-Eastern Mediterranean Health Journal, 2 (2), 315-319, 1996*.

YAHYAA, B. T., AL-SAMARRAI, M. A. M. & JADOO, S. A. A. 2019. Prevalence and perception of women about consanguineous marriage in Al-Ramadi City. *Indian Journal of Public Health Research & Development,* 10.