Kerbala Journal of Pharmaceutical Sciences

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

ORAI1 Genetic Polymorphism and its Correlation with Erythropoietin Resistance, Hemoglobin, and Erythropoietin Serum Level in Iraqi Patients with Chronic Renal Failure on Hemodialysis

Farah F. Alsabbagh[1], Amal Umran Mosa[1], Abo Almaali H.M[1], Hassanain Salah Jafer[2]

1 Pharmacology and Toxicology Department/ Pharmacy College/University of Kerbala

2 Imam Hussain Medical City

Received: 2023/11/12

Accepted: 2023/11/19

Published: 2024/01/04

Keywords: chronic renal failure, ORAI1, genetic polymorphism, hemoglobin, erythropoietin resistance, hemodialysis.

DOI:10.62472/kjps.v14.i23.74-83

Abstract

Introduction: Chronic renal failure CRF is a widespread, irreversible disease that develops gradually and is classified into five stages depending on the glomerular filtration rate GFR. Anemia is a considerable complication of CRF that becomes serious as GFR decreases, erythropoietin-stimulating agent ESA is used for treating anemia, but the resistance to it stands against its goal in many patients. Studying the genetic polymorphism in one of the erythropoietin-activated pathway store-operated calcium channels SOCs gene is a trial for investigating the cause of resistance.

Method: In this study, 174 subjects were included. Among 112 hemodialysis patients with CRF, 54.5% were non-responders, and the enrolled participants ranged from 20 to 79 years; a single nucleotide polymorphism in the ORAI1 gene was selected. Genotyping was done using an allele-specific polymerase chain reaction technique, and the data was analyzed through the Statistical Package for the Social Sciences.

Results: The results showed that hemoglobin levels differ significantly between the genetic groups, while erythropoietin levels showed no significant variation.

Conclusion: ORAI1 genetic polymorphism has no significant role in erythropoietin resistance in Iraqi hemodialysis patients with CRF, but this genetic variation significantly affects hemoglobin levels, depending on this study.

تأثير تعدد الأشكال الجيني لجينَيORAI1 وSTIM1 على مقاومة الارثروبويتين في المرضى العراقيين الذين يعانون من الفشل الكلوي المزمن و المعتمدين على الديلزة الدموية

الخلاصة

المقدمة: يعد الفشل الكلوي المزمن مرض تقدمي يتطور تدريجياً ويتم تصنيفه إلى خمس مراحل بناءً على معدل الترشيح الكبيبي، فقر الدم هو مضاعفة كبيرة للفشل الكلوي المزمن وتزداد خطورته مع انخفاض معدل الترشيح الكبيبي . تستخدم العوامل المنشطة للإريثروبويتين لعلاج فقر الدم ولكن المقاومة لها تعيق فعاليتها لدى العديد من المرضى. تُعد دراسة تعدد الأشكال الجينية في احد جينات قنوات الكالسيوم المعتمدة على المخازن وهي إحدى مسارات تنشيط الإريثروبويتين محاولة لاستكشاف سبب المقاومة.

الطرق: في هذه الدراسة، تم ضم ١٧٤مشاركًا من بينهم ١١٢مريضًا بغسيل الكلى يعانون من الفشل الكلوي المزمن، وكان ٥٤,٥٪منهم غير مستجيبين، وتراوح عمر المشاركين المسجلين بين ٧٩-٢٠عامًا، تم اختيار تعدد الشكل الجيني المفرد في جين ، ORAI1 تم إجراء تحديد النمط الجيني باستخدام تقنية تفاعل البوليميراز المتسلسل النوعي للأليل وتم تحليل البيانات من خلال الحزمة الإحصائية للعلوم الاجتماعية.

النتائج: أظهرت النتائج اختلافًا ملحوظًا في مستويات الهيموجلوبين بين المجموعات الجينية، بينما لم يظهر أي تباين ملحوظ في مستويات الإريثروبويتين.

الإستنتاج : أوضحت هذه الدراسة أن التعدد الشكلي الجيني في جينORAI1 لا يؤثر بشكل كبير على مقاومة الإريثروبويتين لدى مرضى غسيل الكلى العراقيين المصابين بالفشل الكلوي المزمن ، ولكن هذا التباين الجيني له تأثير ملحوظ على مستويات الهيموجلوبين.

1. Introduction

Chronic renal failure CRF is a widespread, irreversible disease that develops gradually and is classified into five stages depending on the glomerular filtration rate GFR. (Ammirati, 2020) one of the complications of CRF is anemia, which contributes to poor clinical outcomes and Increased mortality rate, and as the GFR decreased, anemia became serious (Shaikh, Hashmi and Aeddula, 2019). The most common types of anemia in CRF are Normocytic, normochromic, and hypo-proliferative (Rosita *et al.*, 2021).

As the renal failure progresses, the kidney mass will reduce, and so will the Erythropoietin. Production will decrease because the kidney is the only source of this hormone in adults. (Hayat, Haria and Salifu, 2008) The Food and Drug Administration (FDA) approved the use of the erythropoietin-stimulating agent ESA in 1989 (Wish, 2021). Still, the resistance to ESA stands against treating anemia in many patients (Hanna, Streja and Kalantar-Zadeh, 2021), which can be caused by Iron deficiency, hyperparathyroidism, inadequate dialysis, malnutrition, and chronic inflammation. (Alves *et al.*, 2015) Even when these causes are treated, some patients still exhibit erythropoietin resistance, which needs more explanation.

In 2003, a Retrospective observational study in London was done to investigate the impact of angiotensin-converting enzyme (ACE) polymorphisms on erythropoietin (EPO) requirement and found that patients with the II genotype require higher doses of ESA. (Varagunam *et al.*, 2003) A study in China to predict the association of genetic polymorphism in the DDAH2 gene and erythropoietin resistance in patients on maintenance hemodialysis suggests that complex genetic variations may influence erythropoietin resistance in those patients. (Wang *et al.*, 2017) An observational cross-sectional study done in Saudi Arabia showed that genetic polymorphisms in ACE may affect the response of hemodialysis patients to ESA. (Hamdan Almaeen and Mostafa-Hedeab, 2021) ORAI1 gene was selected for this study because it is one of the store-operated calcium channels (SOCs) genes, as SOCs have a novel role in erythropoiesis (Lee *et al.*, 2019).

Aim of the study: this study was designated to find the impact of ORAI1 genetic polymorphism on erythropoietin resistance, hemoglobin, and erythropoietin serum levels in patients with CRF on hemodialysis in Iraq.

2. Material and Method

2.1 Patients' Constant and Enrollment

This cross-sectional observational study was performed from 11- 2022 to 4- 2023 in Karbala at Imam Al-Hussain Medical City/ Doctor Adel Al Sabbah Center for Hemodialysis. The study protocol was licensed by the Scientific and Ethical Committee of Pharmacy College / Kerbela University; after explaining the nature and purpose of the study, each subject signed an informed consent form.

One hundred seventy-four subjects were included in the study, 112 patients ranging from 20-79 years of age with CRF treated with ESA at the recommended weekly dose and being on hemodialysis and treatment for more than four months, 62 healthy subjects were enrolled as a reference for biochemical tests. Clinical and demographic data were obtained from the subjects with blood samples at enrollment.

These data included gender, age, weight, medical history, family history, and biochemistry data.

2.2 Molecular Analysis

Genomic DNA was extracted from a blood sample as stated by the protocol of gSYNC for blood genomic DNA extraction kit. DNA concentration and purity were measured using a nano-spectrophotometer nanodrop. The DNA purity was measured at the A260/A280 ratio.

The single nucleotide polymorphism (SNP) rs6486795 of the ORAI1 gene was selected, and Prof DR. Hassan Mahmood Musa designed the primers for rs6486795 Snv allele T>A

Allele-specific PCR technique was used to detect the SNP after several trials of PCR to The optimization of PCR was performed to obtain the best concentration of primers and best annealing temperature. Bioneer PCR Premix was used.

2.3 Statistical Analysis

The present study's data was entered and analyzed through the Statistical Package for the Social Sciences (SPSS version 22). The data were presented as frequencies, percentages, mean and standard deviation in appropriate tables and graphs, or mean differences in others. Chi-square test, one-way ANOVA test, and post hoc analysis were used where applicable to find out the possible association between the related variables of the current study, as LSD was used when equal variances were assumed. In contrast, Dunnett's T3 was used when equal variances were not considered depending on Levene's test for homogeneity of variances. Besides, Hardy Weinberg equilibrium was used to detect the prediction of alleles distribution. The statistical association was considered significant when the p-value was equal to or less than 0.05 (P value ≤ 0.05).

3. Results

One hundred twelve patients were included (66 male and 46 female), 51 responders and 61 non-responders; table 1 shows Descriptive statistics for continuous variables of the study, the data presented by means.

Table 1: Descriptive Statistics for Continuous Variables

Variable	Min.	Max.	Mean	Std. Deviation
Age (Year)	22.00	79.00	50.9464	13.42033
Weight (Kg)	33.00	130.00	66.2768	15.74127
Duration of disease (Months)	4.00	180.00	41.8661	41.18525
Duration of dialysis (Months)	4.00	180.00	30.9643	29.17976
Duration of treatment (Months)	4.00	156.00	32.8661	30.10836
Epo mlU/ml	2.49	29.80	13.8574	4.46875
Hb g/dl	6.10	13.10	9.6125	1.79835
BU mg/dl	37.00	214.00	115.9865	34.36958
S. Cr mg/dl	3.50	15.00	7.7296	2.09955

*** [Epo] erythropoietin serum level, [Hb] hemoglobin level, [BU] blood urea, [S. Cr] serum creatinine, [Min] minimum, [Max] maximum, [Std] standard.**

The distribution of ORAI1 gene polymorphism (rs6486795) in different genotypes in this study showed that the TT genotype and TC genotype were almost similar in frequencies, 39.3% and 38.4%, respectively, and the CC genotype was the lesser 22.3% in the 112 enrolled patients as illustrated in fig1 presented by numbers.

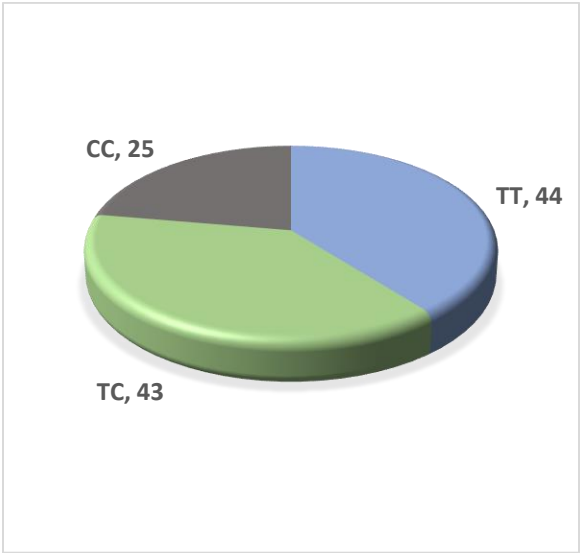

Figure1: Distribution of ORA1 Gene Polymorphism rs6486795 Different Genotypes in Patients

Depending on this study there was no association between gender and genetic variation as shown in Table 2 and no association between the independent variable and response as shown in Table.3

Table 2: Association Between Gender and Genetic Variation

Demographic parameters		Gender	
		Male	Female
Patient Genotype (N=112)	TT N (44)	24	20
	TC N (43)	28	15
	CC N (25)	14	11
P value		0.572	

Hardy Weinberg equilibrium test was done to show the expected frequency of genotype groups, and the expected predominant group will be the hetero TC group based on this study as shown in Fig. 2 which is statistically significant, p-value <0.05.

Table 3: Association Between Independent Variables and Response

Variable		**Responder (No.)**	**Non-Responders (No.)**	**P-value**
Age (Year)	20-39	15	10	0.440 NS
	40-59	29	21	
	60-79	17	20	
Duration of Rx (Months)	4-50	51	39	0.617 NS
	51-90	7	9	
	91-156	3	3	
Duration of Dialysis (Months)	4-60	58	44	0.217 NS
	61-120	2	6	
	121-180	1	1	

[NS]= Non significant

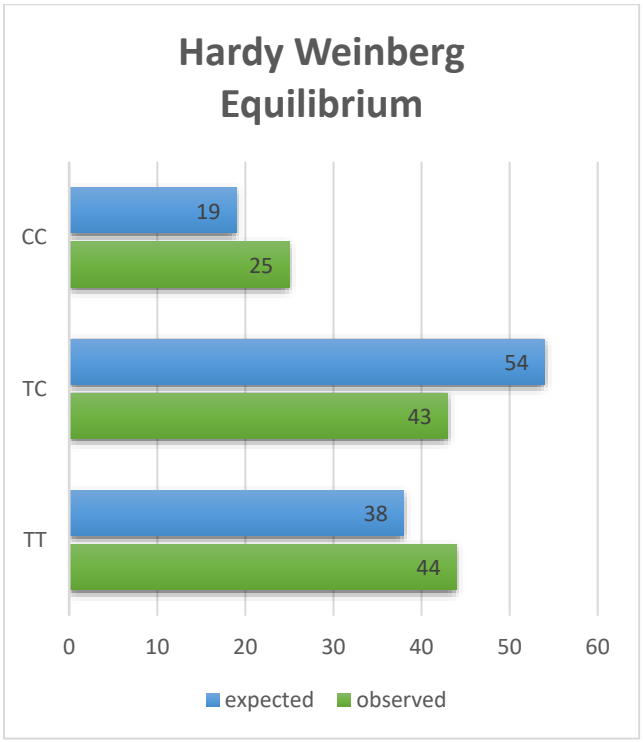

Figure 2: Hardy–Weinberg Equilibrium for ORAI1 Gene (rs6486795) in Patients

Biochemical parameters between genetic groups of patients are summarized in Table.4 presented as mean ± SD showed CC group has a statistically significant rise over TT group in hemoglobin level and also in blood urea, while the control group has a statistically significant difference in hemoglobin level (rise) and serum creatinine (decrease) compared with the genetic groups (p-value <0.05).

Table 4: Mean ± SD of Biochemical Parameters Between Groups of ORAI1 Gene (rs6486795)

Parameters	Groups								P-value
	Control		TT		TC		CC		
	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		
Epo mlU/ml	13.04	3.10	13.52	4.07	14.30	4.70	14.15	4.77	0.362 NS
Hb g/dl	13.48	1.03	9.13	1,69	9.74	1.83	10.23	1.73	0.001 S 0.005 S
BU mg/dl	25.48	7.79	112.97	34.15	112.74	33.63	126.84	35.15	0.001 S CC
S. Cr mg/dl	0.86	0.16	7.81	2.21	7.78	2.22	7.48	1.69	0.01 S control

[Epo] erythropoietin serum level, [Hb] hemoglobin level, [BU] blood urea, [S. Cr] serum creatinine, [S]= Significant, [NS]= Non significant

4. Discussion

This study was done to find the correlation between the resistance of ESA and the genetic polymorphism in the SOCs gene (ORAI1). The results showed that TT and TC genotypes were close in percentage, 39.3% and 38.4%, respectively, while the CC genotype represented 22.3%; by comparing these results with previous studies, some similarities presented, for example, the Taiwanese study in which 290 normal controls were included also found that the two prominent groups were TT and TC (41.72% and 43.45% respectively). The CC group was the lowest in percent(14.83%). (Chang *et al.*, 2014) Another Taiwanese study of 579 chronic kidney disease patients showed the following genetic predisposition: TT genotype 40.7%, TC genotype 47.0%, and CC genotype 12.3%. (Hwang *et al.*, 2014)

Depending on the results of this study, there is no association between gender differences Genetic variations and neither the duration of the dialysis nor the duration of the treatment have a statistically significant effect on the patients' response to ESA. And there is no considerable variation in erythropoietin levels between the genetic groups.

The Hardy-Weinberg equilibrium test represented that the expected results will be a decrease in CC and TT genotypes with an obvious increase in the TC genotype, which is expected to be the prominent group and is statistically significant.

In this study, the CC genotype showed a statistically significant elevation in hemoglobin level over the TT group, which indicates better response; on the other hand, the CC group has a higher BU level than the other groups, representing poor clinical outcomes. This differs from the results of a previous study that was done in 2021, which showed that the CC/TC genotype has a high risk of erythropoietin resistance. (Kao *et al.*, 2021)

5. Conclusion

In ORAI1 genetic polymorphism (rs6486795), the CC genotype may represent the lowest percentage. Furthermore, this group may have a higher Hb level than other groups but may also have negative outcomes. On the other hand, ORAI1 genetic polymorphism has no significant association with erythropoietin resistance in Iraqi patients with CRF on hemodialysis.

Reference

Alves, M. T. *et al.* (2015) 'Resistance of dialyzed patients to erythropoietin', *Revista brasileira de hematologia e hemoterapia*. SciELO Brasil, 37, pp. 190–197.

Ammirati, A. L. (2020) 'Chronic kidney disease', *Revista da Associação Médica Brasileira*. SciELO Brasil, 66, pp. s03–s09.

Chang, W.-C. *et al.* (2014) 'Identifying association model for single-nucleotide polymorphisms of ORAI1 gene for breast cancer', *Cancer Cell International*. Springer, 14, pp. 1–6.

Hamdan Almaeen, A. and Mostafa-Hedeab, G. (2021) 'Haematological Indicators of Response to Erythropoietin Therapy in Chronic Renal Failure Patients on Haemodialysis: Impact of Angiotensin-Converting Enzyme rs4343 Gene Polymorphism', *Pharmacogenomics and Personalized Medicine*. Taylor & Francis, pp. 1055–1068.

Hanna, R. M., Streja, E. and Kalantar-Zadeh, K. (2021) 'Burden of anemia in chronic kidney disease: beyond erythropoietin', *Advances in therapy*. Springer, 38(1), pp. 52–75.

Hayat, A., Haria, D. and Salifu, M. O. (2008) 'Erythropoietin stimulating agents in the management of anemia of chronic kidney disease', *Patient preference and adherence*. Taylor & Francis, pp. 195–200.

Hwang, D.-Y. *et al.* (2014) 'Genetic polymorphisms of ORAI1 and chronic kidney disease in Taiwanese population', *BioMed Research International*. Hindawi, 2014.

Kao, C.-C. *et al.* (2021) 'The role of genetic polymorphisms in STIM1 and ORAI1 for erythropoietin resistance in patients with renal failure', *Medicine*. LWW, 100(17), p. e25243.

Lee, Y. Y. *et al.* (2019) 'Store-operated Ca2+ Channels Are Involved In Erythropoiesis'. The American Society for Cell Biology.

Rosita, L. *et al.* (2021) 'Type of anemia correlate with ferritin levels in patients with Chronic Kidney Disease (CKD): a cross sectional study', *Bali Medical Journal*, 10(3), pp. 867–871.

Shaikh, H., Hashmi, M. F. and Aeddula, N. R. (2019) 'Anemia of chronic renal disease'.

Varagunam, M. *et al.* (2003) 'Angiotensin-converting enzyme gene polymorphism and erythropoietin requirement', *Peritoneal dialysis international*. SAGE Publications Sage UK: London, England, 23(2), pp. 111–115.

Wang, T. *et al.* (2017) 'Association of the− 449GC and− 1151AC polymorphisms in the DDAH2

gene with asymmetric dimethylarginine and erythropoietin resistance in Chinese patients on maintenance hemodialysis', *Clinical and Experimental Pharmacology and Physiology*. Wiley Online Library, 44(9), pp. 961–964.
Wish, J. B. (2021) 'Treatment of anemia in kidney disease: beyond erythropoietin', *Kidney International Reports*. Elsevier, 6(10), pp. 2540–2553.