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Impact of CYP3A4 rs2242480 Gene Polymorphism on Rivaroxaban Plasma Level Concentration and Bleeding Risks among Iraqi Patients with Atrial Fibrillation

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Abstract

Background: A prevalent cardiac arrhythmia that dramatically raises the risk of stroke and systemic embolism is atrial fibrillation (AF). Moreover, because of their known pharmacokinetic and pharmacodynamic profile, convenience of administration, and fixed-dose administration, anticoagulants, such as rivaroxaban, are important and widely used to treat atrial fibrillation and prevent stroke. However, various factors, including genetic variations in the drug's metabolism, may have a role in the variation responsible for the response's interindividual heterogeneity. Cytochrome P450 3A4 is the major enzyme responsible for the metabolism of rivaroxaban. So, the polymorphisms on this enzyme (such as rs2242480) may change the metabolism, and then a change in plasma concentration may increase or decrease bleeding events.

Study objectives: This study aims to determine the frequency of the CYP3A4 single-nucleotide polymorphism (SNP) rs2242480 in this cross-sectional study to examine the effect of the polymorphism on the plasma concentration of rivaroxaban and risk of bleeding in patients with atrial fibrillation (AF) from Iraq

Patients and Methods: This study examined the effect of the CYP3A4 rs2242480 polymorphism on the rivaroxaban plasma concentration levels in 100 Iraqi atrial fibrillation patients receiving a fixed dose of 20 mg/day. Genotyping was done with allele-specific PCR, and the plasma levels were determined with HPLC.

Results: The genotype distributions for rs2242480 were 10% TT, 29% CT, and 61% CC (wild-type). The mean rivaroxaban concentration was greater in patients with the T allele (CT and TT) (341.60 (336.80) ng/mL for CT and 399.00 (349.78) ng/mL for TT) than in patients with the CC genotype (152.60 (344.40) ng/mL). Still, this difference was not statistically significant ($p = 0.248$). Clinical and biochemical indicators did not significantly correlate with genotype, so bleeding events were reported in 10% of patients; there was also no significant association between bleeding events and CYP3A4 rs2242480 genotype. The bleeding episodes were generally mild and did not result in significant complications.

Conclusion: In Iraqi individuals with atrial fibrillation, the CYP3A4 rs2242480 polymorphism has no recognized effect on rivaroxaban plasma levels or bleeding episodes. These findings imply that rivaroxaban treatment in this cross-sectional study may not require routine genotyping for this variant. Further extensive research and genetic analyses are needed to validate these results and investigate additional genetic factors impacting rivaroxaban metabolism.

تأثير تعدد أشكال جين CYP3A4 rs2242480 على مستوى تركيز ريفاروكسابان في البلازما ومخاطر النزيف بين المرضى العراقيين المصابين بالرجفان الأذيني

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الملخص

الخلفية: يعرف الرجفان الأذيني (AF) بانه من أكثر اضطرابات القلب شيوعًا، حيث يزيد بشكل كبير من خطر الإصابة بالسكتة الدماغية والانسداد الجهازي. علاوة على ذلك، تعتبر مضادات التخثر، مثل ريفاروكسابان، مهمة وواسعة الاستخدام لعلاج الرجفان الأذيني والوقاية من السكتة الدماغية، نظرًا لملفها الدوائي والدوائي الحيوي المعروف، وسهولة تناولها، وتناولها بجرعات ثابتة. ومع ذلك، قد تلعب عوامل مختلفة، بما في ذلك التغايرات الجينية في استقلاب الدواء، دورًا في التباين المسؤول عن تباين الاستجابة بين الأفراد. إن إنزيم السيتوكروم P450 3A4 هو الإنزيم الرئيسي المسؤول عن استقلاب ريفاروكسابان. لذا، قد يؤدي تعدد الأشكال في هذا الإنزيم) مثل (rs2242480 إلى تغيير في الاستقلاب، مما قد يؤدي إلى تغيير في تركيز البلازما وزيادة أو تقليل أحداث النزيف.

أهداف الدراسة: هدفت هذه الدراسة إلى تحديد تكرار تعدد الأشكال أحادي النوكليوتيد CYP3A4 rs2242480 (SNP) في هذه الدراسة المقطعية لفحص تأثير هذا التعدد على تركيز ريفاروكسابان في البلازما ومخاطر النزيف لدى المرضى المصابين بالرجفان الأذيني (AF) من العراق.

المرضى والطرق: درست هذه الدراسة تأثير تعدد الأشكال CYP3A4 rs2242480 على مستويات تركيزعقار ريفاروكسابان في البلازما لدى 100 مريض عراقي مصاب بالرجفان الأذيني يتلقون جرعة ثابتة قدرها 20 ملغ يوميًا. تم إجراء تحليل الجينات باستخدام PCR الخاص بالأليلات، وتم تحديد مستويات البلازما باستخدام.HPLC

النتائج: كانت التوزيعات الجينية لـ rs2242480 هي 10%TT ، 29%CT ، و 61% CC(النوع البري). كان متوسط تركيز ريفاروكسابان أعلى في المرضى الذين يحملون الأليل CT) T و (336.80) 341.60) (TTنانوغرام/مل لـ CT و399.00 (349.78) نانوغرام/مل لـ (TT مقارنة بالمرضى الذين يحملون الاليل CC (152.60 (344.40) نانوغرام/مل). ومع ذلك، لم يكن هذا الاختلاف ذا دلالة إحصائية .($p = 0.248$) لم تتوافق المؤشرات السريرية والبيوكيميائية بشكل كبير مع الجينوتيب، وعلى الرغم من الإبلاغ عن أحداث النزيف في 10% من المرضى؛ لكن لم يكن هناك ارتباط كبير بين أحداث النزيف والتوزيع الجيني .CYP3A4 rs2242480 كانت حالات النزيف عمومًا خفيفة ولم تؤد إلى مضاعفات كبيرة.

الاستنتاج: في الأفراد العراقيين المصابين بالرجفان الأذيني، لم يكن هناك تأثير معروف لتعدد أشكال CYP3A4 rs2242480 على مستويات ريفاروكسابان في البلازما أو أحداث النزيف. تشير هذه النتائج إلى أن علاج ريفاروكسابان في هذه الدراسة المقطعية قد لا يتطلب تحليل الجينات الروتيني لهذا المتغير. هناك حاجة إلى مزيد من الأبحاث الشاملة والتحليلات الجينية للتحقق من هذه النتائج واستكشاف عوامل جينية إضافية تؤثر على استقلاب ريفاروكسابان.

1. Introduction

Atrial fibrillation (AF) is a highly prevalent and risk-provoking cardiac arrhythmia associated with stroke and systemic embolization (Kostopoulos & Effraimidis, 2024). Atrial fibrillation (AF) is frequently an indication for direct oral anticoagulants (DOACs). Rivaroxaban is one of the DOACs that has been extensively used due to the fact that rivaroxaban has several advantages over VKA, warfarin (Hindley et al., 2023), such as predictable pharmacokinetics, fixed dosing, and not requiring regular monitoring. The manuscript also reports that rivaroxaban is known to decrease thromboembolic events and directly inhibit factor Xa and thrombin generation in NVAF patients (Ferro et al., 2020; Kearon et al., 2016). Nevertheless, it works well. There are some biological, genetic differences in response to drugs, and some of them center around drug metabolism. The elimination of rivaroxaban is mediated to a large extent by the cytochrome P450 3A4 (CYP3A4) enzyme. Things like rs2242480 could then affect enzyme activity and thus rivaroxaban PK. Of interest is the variant rs2242480 (C>T exchange), which may lead to variable plasma concentrations of rivaroxaban since individuals carrying the T allele would have higher drug levels. That may increase the risk of bleeding, a common side effect of anticoagulant medications. As research has shown that CYP3A4 polymorphism, including CYP3A4 rs2242480, can affect both the efficacy and safety of rivaroxaban, pharmacogenetic genotyping has a potential value in the individualization of treatment of OAC agents (Chadha et al., 2022). Optimizing the use of rivaroxaban in AF patients is critical for reducing the incidence of S. The effect of such polymorphisms, including rs2242480, on the plasma concentrations of rivaroxaban has not been elucidated. However, these data could be used for individualized treatment, since the mutant CYP3A4 alleles might be involved in drug metabolism (X. Li et al., 2024). The present work aimed to investigate the potential link between rs2242480 polymorphism, the pharmacokinetics of rivaroxaban, and bleeding in Iraqi patients with atrial fibrillation, which may personalize management in everyday practice.

2. Materials and Methods

2.1 Study Design

A total of 100 Iraqi patients with atrial fibrillation were enrolled in the trial and received 20 mg/day of rivaroxaban. Enrollment criteria included age of 18–80 years and at least 3 months on rivaroxaban treatment. Exclusion criteria included patients treated with other anticoagulants or with severe liver or kidney failure, uncontrolled hypertension

2.2 Blood Sample Collection

DNA from blood was isolated from the patient using the isolation kit Geneaid from a blood sample after blood sampling and performing biochemical analysis, e.g., INR, PT. The concentration and purity of the DNA samples were determined by spectrophotometer analysis, and high-quality samples were selected for further genetic analyses. In addition, the concentration of rivaroxaban in the plasma samples was analyzed with HPLC.

2.3 CYP3A4 rs2242480 genotyping

CYP3A4 rs2242480 was genotyped using the allele-specific PCR (AS-PCR). This technique confirmed the rs2242480 polymorphism C and T alleles. PCR amplification was performed for the CYP3A4 gene fragment containing the polymorphisms. The PCR products were run for separation, and the genotype of each patient was established by agarose gel electrophoresis. Information on the primers used in our study is presented in Table1. This table displays important information about primers used in the genetic analysis process, such as sequences and the length of the primers. The primers were custom-designed by a specialized laboratory in Karbala Province using Primer-BLAST software and were procured as lyophilized products from Macrogen, Korea, at various picomole concentrations. The lyophilized forward and reverse primers were reconstituted in nuclease-free water.

Table1: Shows the Primer Set Tubes' Nucleotide Sequence

Primer set tubes	Nucleotide sequence	Product length
rs2242480 Forward primer C allele Forward primer T allele Reverse primer common	 5-CCTCCCTCCTTCTCCATGTAC-3 5-CCTCCCTCCTTCTCCATGTAT-3 5-TGCGGAAATGCTCTCTCTGG-3	488

2.4 Measurement of Rivaroxaban Plasma Concentrations

Rivaroxaban plasma levels were measured with a C18 column in high-performance liquid chromatography (HPLC). Diluted plasma samples were sonicated and filtered after treatment with a mobile phase of acetonitrile and water. The association between rivaroxaban levels in plasma and genotype was assessed for each genotype, and rivaroxaban levels were measured by retention time. (Derogis et al., 2017)

2.5 Clinical Outcome Assessment

Clinical information was gathered from patient medical records, including bleeding occurrences. Additionally, PT and INR measurements are used as indicators for bleeding occurrences.

2.6 Statistical Analysis

The study utilized (IBM SPSS version 26) for data analysis, presenting results in tables and graphs as means, standard deviations, frequencies, and percentages. Independent t-tests and ANOVA with post hoc analysis were applied for normally distributed data to assess relationships between variables. Kruskal-Wallis tests were used for non-normally distributed data, along with Bonferroni correction, with significance set at $p < 0.05$. A multiple-choice test assessed the percentage of symptom reactions.

3. Results

3.1 Demographic and Clinical Characteristics

A total of 100 patients were included in this study, versus the 100 Iraqi patients treated with rivaroxaban in this trial, who had atrial fibrillation and were receiving 20 mg of rivaroxaban in this study. The sample composition presented more females (55%) than males (45%), and 49% of participants were over 60. Concerning BMI, 52% were obese and 35% were overweight, with 36% having diabetes and 85% having hypertension, both of which are significant atrial fibrillation risk

factors. These demographic characteristics are relevant in interpreting the potential impact of age, body weight, and other factors on treatment response to rivaroxaban. According to Table2.

Table2: Descriptive Statistics of Demographic Characteristics of The Studied Patients (N=100)

Variable		N	Percent
Sex	Male	45	45
	Female	55	55
Age	30-45	9	9
	46-60	42	42
	>60	49	49
BMI	Underweight	0	0
	Normal	13	13
	Overweight	35	35
	Obese	52	52
Duration of treatment (months)	3-42	85	85
	>42	15	15
Diabetes mellitus	Yes	36	36
	No	64	64
Hypertension	Yes	85	85
	No	15	15

3.2 Genotype Distribution of CYP3A4 rs2242480

The study's cross-sectional CYP3A4 rs2242480 genotype distribution was as follows: 10% TT (homozygous minor allele), 29% CT (heterozygous), and 61% CC (wild-type). Age, sex distribution, and body mass index did not significantly differ among genotype groups ($p > 0.05$), suggesting that the population is homogeneous regarding fundamental demographics. The mean rivaroxaban concentration was greater in patients with the T allele (CT and TT) (341.60 (336.80) ng/mL for CT and 399.00 (349.78) ng/mL for TT) than in patients with the CC genotype (152.60 (344.40) ng/mL). Statistical analysis failed to show significance ($p = 0.248$), indicating a trend rather than a clear-cut effect, even though carriers of the T allele had greater mean plasma levels. So, the rs2242480 polymorphism was not associated with changes in these clinical parameters in this cross-sectional study. Table3 shows the genotype distribution for the single-nucleotide polymorphism rs2242480 in 100 patients. The three genotypic types found were TT (homozygous mutant), CT (heterozygous), and CC (wild type).

Table 3: Distribution of Gene Polymorphism for Rs2242480 Genotype in Patients (N = 100)

Variable		Frequency	Percent
Genotype SNP1	CC wild	61	61
	CT hetero	29	29
	TT homo	10	10

3.3 Rivaroxaban Plasma Concentrations and genotype of rs2242480

Rivaroxaban plasma levels were collected for all patients. The rivaroxaban concentration was slightly higher in TT genotype patients than CC genotype patients; however, this was not statistically significant ($p > 0.05$). Table 4 displays the information about the relationship between the concentration of rivaroxaban and the genotype of rs2242480 as follows: Rivaroxaban concentration was greater in patients with the T allele (CT and TT) (341.60 (336.80) ng/mL for CT and 399.00 (349.78) ng/mL for TT) than in patients with the CC genotype (152.60 (344.40) ng/mL).

Table 4: Plasma Concentration of Rivaroxaban By Genotypes

SNP	Genotype	Plasma Concentration (ng/mL)	P-value
rs2242480	CC	152.60 (344.40)	0.248
	CT	341.60 (336.80)	
	TT	399.00 (349.78)	
Kruskal-Wallis test was used, with a significant p-value of less than 0.05. Results are presented as the median with the IQR			

3.4 Bleeding Events and rs2242480 Genotypes

The study looks at the relationship between rs2242480 and patient bleeding events. Table5 shows that 10% of patients experienced bleeding episodes, although there was no significant correlation between bleeding events and the CYP3A4 rs2242480 genotype. CC, CT, and TT genotypes of rs2242480 exhibit different proportions of hemorrhagic and non-hemorrhagic cases. However, statistical analysis shows no significant relationship between bleeding risk and these genotypes ($p = 0.940$). This suggests that these genetic differences had no discernible impact on hemorrhagic complications in the population under study. Additionally, Table 6, a study of the relationship

between rs2242480 and the laboratory biomarkers INR and PT used to identify bleeding events, also reveals no discernible impact.

Table5: Bleeding Event and rs2242480

SNP	Genotype	Non-Hemorrhage	Hemorrhage	P value
rs2242480	CC	56	5	0.940
	CT	26	3	
	TT	9	1	

Table 6: List of The Laboratory Biomarkers for Patients Based On Rs2242480

Parameter	CC		TT	MC	P value
INR	1.08(0.30)	1.23(0.20)	1.27(0.25)	NS	0.090
PT	12.85(2.75)	14.00(1.50)	13.70(2.80)	NS	0.152
Kruskal-Wallis test, where a p-value less than 0.05 was considered significant. The median with IQR					

4. Discussion

The present study aims to assess the impact of the rs2242480 gene polymorphism of CYP3A4 on plasma concentration of rivaroxaban and the occurrence of bleeding events in Iraqi patients with atrial fibrillation (AF). In the present study, it was also demonstrated that the rs2242480 polymorphism does not influence the rivaroxaban plasma level or bleeding events. That is distinct from a previous study, which showed that changes in the gene CYP3A4, a major factor in drug metabolism, could change the way drugs are processed (X. Li et al., 2024). Introduction The cytochrome P450 isoenzyme CYP3A4 is a key enzyme in the metabolism of rivaroxaban and other drugs. The rs2242480 and other CYP3A4 polymorphisms have also been found in a prior study to be associated with the pharmacokinetics of rivaroxaban, thereby affecting its plasma concentration and therapeutic response (Chadha et al., 2022). The concentration of rivaroxaban among the three genotypes of rs2242480 was different but was not statistically significant in this research. This means that SNP rs2242480 has no effect on rivaroxaban metabolism among the Iraqi AF patients investigated in the present study. These results from the present study confirm the previous negative results and also found no association between drug plasma levels of rivaroxaban in special populations and CYP3A4 polymorphism. (Mueck et al., 2013). For example, CYP3A4 metabolizes rivaroxaban, and the rs2242480 locus is unlikely to have a marked effect on the clearance or plasma concentration of rivaroxaban. Furthermore, an examination also showed that polymorphisms such as rs2242480 might not contribute to one ethnicity; hence, the impact of genetic polymorphisms in rivaroxaban metabolism is possibly ethnically related (Perzborn et al., 2010). Similarly, no association was observed between polymorphism rs2242480 and bleeding

risk in patients undergoing rivaroxaban therapy. This is remarkable, as bleeding complications constitute a significant issue in patients under anticoagulant treatment. Moreover, former studies also mention that the high rivaroxaban plasma level is a risk for bleeding (Cepeda et al., 2025). Although genotypic variety was present in this cohort, it was not related to the bleeding events, and there was no association of the drug levels with bleeding events. This indicates that the bleeding tendency may be influenced by the spectrum of various factors, including comorbidity, co-medication, and other background genetic factors, rather than that of rs2242480 in CYP3A4 alone. Therefore, owing to these, the rs2242480 mutation could not be requested to be tested in Iraqi atrial fibrillation patients receiving rivaroxaban. However, it is essential and has profound therapeutic management. Although CYP3A4 continues to be the principal isoenzyme for rivaroxaban metabolism, these other variables may be better predictors of plasma levels and risk of bleeding (Kearon et al., 2016). These results should be confirmed in larger, more diverse populations, and other genetic variants that might influence rivaroxaban pharmacokinetics and outcomes should be explored. Finally, because of rivaroxaban's complex metabolic characteristics, a number of environmental and genetic parameters could play a role in such inter-individual treatment response differences.

5. Conclusion

This study sought to determine the frequency and the effects of the CYP3A4 (rs2242480) polymorphism on plasma rivaroxaban concentration and the risk of bleeding among Iraqi atrial fibrillation patients. The effect of the rs2242480 polymorphism on rivaroxaban plasma concentration was not observed. The concentration of drugs differed to a small degree among carriers with different genotypes. However, these differences were not statistically significant, and rs2242480 would not, therefore, appear to play a large role in modulating the metabolism of rivaroxaban within this population. Moreover, no significant association was observed between rs2242480 and the risk of bleeding in rivaroxaban-treated patients with atrial fibrillation. So, this indicates that this genetic variation has no appreciable effect on rivaroxaban-induced bleeding episodes in the target population. These results suggest that the rs2242480 variant of the CYP3A4 gene was not an essential biomarker of the plasma concentration of rivaroxaban or bleeding risk in Iraqi patients with atrial fibrillation. Furthermore, this variant may not require routine genetic testing to direct the rivaroxaban prescription in such a patient population. These results need to be validated, and other potential genetic factors that affect the pharmacokinetics and therapeutic effect of rivaroxaban should be explored with a larger sample size and more diverse populations in the future.

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