DOI: https://doi.org/10.31351/vol31iss2pp251-259

Correlation Between CYP2C19 Polymorphisms and Recurrent Risk in Patients with Ischemic Stroke Treated with Clopidogrel in Kurdistan Region-Iraq

Hind S. Jardaq*, [1] and Mohammed Y. Jamal**

* Department of Clinical Pharmacy, College of Pharmacy, Mosul University, Mosul, Iraq.
** Department of Clinical Pharmacy, College of Pharmacy, University of Baghdad , Baghdad, Iraq.

Abstract

Clopidogrel is a prodrug that must be transformed into an active metabolite by hepatic cytochrome P450 (CYP) isoenzymes in order to be active in preventing platelet clotting. Polymorphisms of the CYP2C19 gene can cause a reduction or complete loss of CYP2C19 enzyme activity resulting in inhibiting clopidogrel metabolism, effectiveness and increment of stroke recurrence risk in ischemic stroke patients. This study aims to investigate the correlation between genetic polymorphisms in CYP2C19*2 and*3 and recurrent risk in patients with ischemic stroke taking clopidogrel 75mg in Kurdistan region–Iraq. This retrospective case-control study was carried out at Kurdistan, Erbil, Medicina medical center, and Rizgary general hospital from January 2021 to August 2021. The blood sample was taken from the participants and tested for genotyping. The collection of retrospective data was done by reviewing patients' medical files in the Rizgary hospital and patients' electronic records in the neurology clinic of Medicina medical center. Sixty patients participated, (34) were male and (26) were female, with age range (38-96) years, diagnosed with ischemic stroke in not more than two years and on 75 mg clopidogrel maintenance dose. Genotyping analysis showed that 61.7 % were homozygotes for wild allele *1, 26.7% *(*1/*2)* and *6.7 % (*1/*3)* heterozygotes genotype. The homozygotes for mutant alleles *CYP2C19*2,*3* were 3.3 % *(*2/*2)* and 1.7 % *(*3/*3)* respectively. The (*2/*3)* was not detected in the study population. Results revealed a significant correlation between the risk of stroke recurrence with the existence of variant allele *CYP2C19 *2*, and angiotensin converting enzyme inhibitors/angiotensin receptor blockers (ACEIs/ARBs) usage (P = 0.024, P = 0.039, P = 0.24 respectively). On the other hand, there was no significant relationship between the risk of stroke recurrence and carrying the variant allele *CYP2C19 *3* (P = 1.000). In conclusion, survivors of ischemic stroke treated with clopidogrel who carry CYP2C19*2 allele had a higher risk of recurrent stroke as it is associated with reduced metabolic activity of CYP2C19 enzyme leading to reduction of clopidogrel metabolism and bioavailability.

Keywords: CYP2C19, Gene polymorphism, Clopidogrel, Stroke.

العلاقة بين تعدد صيغ CYP2C19 و خطورة الاصابة بجلطة ثانية في المرضى الذين يعانون من السكتة الدماغية ويتناولون عقار كلوبيدوجرل في إقليم كردستان العراق

هند سالم جردق*[1] و محمد ياوز جمال**

*فرع الصيدلة السريرية ، كلية الصيدلة ، جامعة الموصل، الموصل، العراق.
** فرع الصيدلة السريرية ، كلية الصيدلة ، جامعة بغداد، بغداد ، العراق.

الخلاصة

الكلوبيوجريل هو دواء أولي يجب تحويله إلى مستقلب نشط بواسطة إنزيمات متوازنة السيتوكروم CYP P450 لمنع تجلط الصفائح الدموية. يمكن أن يتسبب تعدد أشكال الجين CYP2C19 في تقليل نشاط إنزيم CYP2C19 أو فقده تمامًا مما يؤدي إلى تثبيط استقلاب الكلوبيدوجريل ، تقليل فعاليته وزيادة خطرتكرار الاصابة بالسكتة الدماغية للمصابين بها لاول مرة. تهدف الدراسة إلى التحقق من العلاقة بين تعدد الأشكال الجينية في 2 * CYP2C19 و3* CYP2C19 و خطر الاصابة بجلطة ثانية في مرضى السكتة الدماغية الذين يتناولون عقار كلوبيدوجريل ٧٥ مجم في إقليم كردستان العراق. أجريت هذه الدراسة بأثر رجعي في كردستان ، أربيل ،مركز ميديسينا الطبي ومستشفى رزكاري العام من يناير ٢٠٢١ إلى أغسطس٢٠٢١ تم أخذ عينات الدم من المشاركين واختبارها من أجل التنميط الجيني. اخذت البيانات من السجلات الطبية للمرضى في المستشفى والسجلات الطبية الإلكترونية للمرضى من عيادة طب الاعصاب .شارك ستون مريضا ، (٣٤) من الرجال و (٢٦) من النساء, تتراوح اعمارهم بين (٣٨-٩٦) سنة. تم تشخيص هؤلاء المرضى بسكتة دماغية لمدة لا تزيد عن عامين ، مع تناول عقار كلوبيدوجريل ٧٥ ملغ.أظهر تحليل التنميط الجيني أن ٦١.٧٪ كانت متجانسة الزيجوت للأليل الطبيعي * ١ ، متغايرة الزيجوت مقسمة إلى ٢٦.٧٪ (* ١ / * ٢) و ٦.٧٪ (* ١ / * ٣)، بينما متجانسة الزيجوت للأليلات الطافرة 3*,2*CYP2C19 ،موزعةالى ٣.٣٪ (* ٢ / * ٢) و ١.٧٪ (* ٣ / * ٣). لم يتم الكشف عن (* ٢ / * ٣) في مجتمع الدراسة. وجدت علاقة بين خطر الاصابة بسكتة دماغية ثانية مع حمل الأليل المتغير 2 * CYP2C19 ، انخفاض نشاط التمثيل الغذائي لإنزيم CYP2C19 واستخدام ACEIs / ARBs (P=٠.٠٢٤) ، P = ٠.٠٣٩ ، P = ٠.٠٢٤على التوالي). من ناحية أخرى ، لم تكن هناك علاقة ذات دلالة إحصائية بين خطر الاصابة بجلطة ثانية وحمل الأليل المتغير (3* CYP2C19=P١).خطرالإصابة بالسكتة الدماغية المتكررة كان اعلى لدى مرضى الجلطة الدماغية الذين يتناولون الكلوبيدوجريل ويحملون 2 * CYP2C19 لأنها مرتبطة بانخفاض النشاط الأيضي لإنزيم CYP2C19 مما يؤدي إلى تقليل فعالية عقار كلوبيدوجريل.

الكلمات المفتاحية: CYP2C19 ، تعدد الأشكال الجيني ، كلوبيدوجريل ، السكتة الدماغية

[1]Corresponding author E-mail: hind.gardaq@yahoo.com
Received: 8/10 /2021
Accepted: 30/1 /2022

Introduction

Stroke is a leading cause of long-term disability and death globally [1]. Ischemic stroke is defined as a brain, spinal cord, or even retinal infarction [2] produced by a blood vessel obstruction due to thrombosis or embolism, resulting in impaired blood supply to the part of the brain fed by this vessel and loss of neurological function [3] Nearly half of ischemic stroke or transient ischemic attack (TIA) patients are at high risk of recurrent stroke within a few months of the initial episode [4]. In the United States, one in every six people will have a stroke throughout their lifetime. More than 13.7 million people suffer a stroke each year, and 5.8 million people die as a result. Over 80 million people have lived through a stroke [1]. Ischemic stroke accounts for roughly 70% of all strokes, with hemorrhagic stroke accounting for the remaining 30%. [5]. Cardio-embolism and large artery atherosclerosis are the most common known causes of ischemic stroke [6]. Clopidogrel, a P2Y12 receptor inhibitor, exhibits higher efficacy, with no added risk of adverse effects, to aspirin in reducing the risk of ischemic stroke, myocardial infarction (MI) and vascular mortality when clopidogrel is added to aspirin [7,8]. When compared to antiplatelet monotherapy, dual antiplatelet therapy (DUAT), a combination of aspirin plus clopidogrel is more effective in reducing the risk of ischemic vascular events [9]. Clopidogrel is an inert prodrug that must be activated in the liver through a complicated biochemical process. About half of the dose of clopidogrel is absorbed from the intestine after oral intake [10]. The carboxylesterase enzyme hydrolyzes over 85% of the absorbed dose of clopidogrel, turning it into an inactive metabolite. The remaining 15% of the dose is transformed into the active metabolite 2-oxoclopidogrel active metabolite in a two-step bio-activation process by several cytochromes P450 enzymes [11-13]. As a result, only 2% of the clopidogrel dose is converted into an active metabolite and enters the systemic circulation [14]. CYP2C19 is responsible for 44.9 percent of clopidogrel conversion to 2-oxoclopidogrel and roughly 20% of active thiol metabolite synthesis from 2-oxoclopidogrel. As a result, CYP2C19 is required for both clopidogrel activation steps [15].

Pharmacogenetics is the field that studies how genetic variation affects individuals' response to drugs. This inter-individual variation can range from inadequate therapeutic efficacy to serious, potentially life-threatening adverse drug reactions [16]. The CYP2C19 gene is located within a cluster of cytochrome P450 genes on chromosome 10q24 containing 8 introns and 9 exons[15]**.** The CYP2C19 gene was expressed in the liver to synthesize a catalytically active enzyme CYP2C19 that is a primary enzyme involved in the conversion of the antiplatelet clopidogrel to the active 2-oxo metabolite [17]. Currently, there is around 25 different type of CYP2C19 mutations, with CYP2C19*2 and CYP2C19*3 being the most prevalent mutant alleles (11). The frequency of these mutations varies depending on trace or ancestral origin, with Asians having a higher frequency than Caucasians and African Americans [2]. The two CYP2C19 mutant alleles CYP2C19*2 and *3 are the key loss of function (LOF) alleles that can cause a reduction or complete loss of CYP2C19 enzyme activity resulting in inhibiting clopidogrel metabolism [11]. Individuals can be classified into three phenotypes based on the number of LOF alleles they carry: non-carriers or Extensive Metabolizers (EMs; *1/*1), patients with one LOF allele or Intermediate Metabolizers (IMs; *1/*2 and *1/*3), and patients with two LOF alleles or Poor Metabolizers (PMs; *2/*2, *2/*3, and *3/*3) (18). Reduced development of the active clopidogrel metabolite and reduced clopidogrel effectiveness was observed in people who have one or two nonfunctional CYP2C19 alleles. The presence of a nonfunctional CYP2C19 allele has been linked to an increased risk of cardiovascular events in numerous studies [2].

This study aims to assess the correlation between genetic polymorphisms in CYP2C19*2 and*3 and recurrent risk in patients with ischemic stroke on daily 75 mg clopidogrel tablets in Kurdistan region–Iraq.

Methods

Patients and study design

From January 2021 to August 2021, an observational mixed retrospective and prospective case-control study was conducted in Erbil, Kurdistan, in two medical centers: the first is the neurology clinic in Medicina medical center, and the second is the neurology ward in Rizgary hospital, one of Erbil's largest hospitals, in collaboration with a neurologist who served as project supervisor. Sixty patients of convenient sample were chosen after meeting the particular inclusion and exclusion criteria. This study's sample size was comparable to that of Alhazzani in 2017, a study conducted in a neighboring country, Saudi Arabia which included fifty patients divided equally into two groups: clopidogrel responders and clopidogrel non-responders [15]. These patients were diagnosed with ischemic stroke for at least two years, taking a 75 mg clopidogrel daily maintenance dose. Patients' blood samples were gathered and examined for genotyping by polymerase chain reaction (PCR) searching for the presence of CYP2C19*2 and *3 polymorphisms ; Patients were divided into two groups based on the presence of stroke recurrence: 30 patients with recurrent stroke (case group) and 30 patients without recurrent stroke (control group). The distribution inside each group is dependent on the existence of polymorphisms (CYP2C19*2 and *3 loss of

function) according to PCR test and arranged into those with mutant genes and those without the mutant gene. The collection of data was done by reviewing the medical files in the hospital and from patients' electronic medical records in the neurology clinic. The demographic factors of patients were chosen according to the risk factors stated in the American heart association/American stroke association (AHA/ASA) guidelines for the prevention of stroke in patients with ischemic stroke or transient ischemic attacks such as age, gender, race, and family history, weight, diabetes mellitus, hypertension, hyperlipidemia (high LDL-cholesterol levels), ischemic heart disease (IHD) and current smoking.The use of cardio-protective medications (statins, angiotensin-converting enzyme inhibitors/angiotensin receptor antagonist [ACEI/ARB], beta-blocker, calcium antagonist, and aspirin) might play an important role in secondary stroke prevention [19], therefore they also were registered with the risk factors in a patient information sheet to be correlated with recurrence of the stroke. As well, the Clopidogrel drug brand is selected as one of the patient demographic factors that can affect the risk of ischemic stroke recurrence based on that modifying the drug salt formulation could change its physicochemical properties, thereby affecting clinical efficacy and safety [20]. In addition, the quality of the clopidogrel drug brand is may be different from that of generic one as it can be affected by the level of impurities present in many copies of the original drug [21].

Inclusion criteria

Patients who are diagnosed with ischemic stroke and taking 75 mg clopidogrel as a daily maintenance dose were included in the study.

Exclusion criteria

1. Patients who are taking drugs interacting with clopidogrel for example tricyclic antidepressants (TCA_s), antipsychotics, warfarin, and glycoprotein IIa/IIIb antagonists.
2. Patients with a history of alcohol or drug abuse.
3. Patients with clotting or other blood disorders.
4. Patients with liver and kidney diseases.

Instruments and Procedure

Molecular analysis

Two milliliters of venous blood were collected from each participant in a 3 ml ethylene diamine tetra acetic acid evacuated tube (EDTA). Samples were stored at -20º before sending for genomic isolation. Genomic DNA isolation from white blood cells was done by using the solid-phase DNA extraction method with a kit provided by Promega (ReliaPrep ™ Blood gDNA Miniprep System). The quality of extracted DNA was checked using ethidium bromide stained 0.5% agarose gel electrophoresis and visualized by UV light.

Polymerase chain reaction and DNA sequencing

The CYP2C19*2 and *3 polymorphisms were detected by the Tetra ARMS (Amplification Refractory Mutation System) polymerase chain reaction method which uses the thermal cycler (Biometra TAdvanced 384 G, 230 V) and four primers (forward and reverse outer primers, forward and reverse inner primers) for amplification. This method is of acceptable specificity because the inner primers designed to be allele-specific. The amplified products of each reaction were separated on 1.5% agarose gel. Automated Sanger sequencing was performed to confirm the different allelic variants of CYP2C19*2 and CYP2C19*3. Sequencing of the purified products was conducted with the sequencing kit according to the instructions of the manufacturer (3700 The BigDye® Terminator v3.1 Cycle Sequencing kit, Applied Biosystems, USA). The sequencing was performed on ABI PRISM 3700 genetic analyzer from Applied Biosystems. The sequencing results were analyzed by FINCH program sequencing analysis software.

Statistical analysis

Continuous data were expressed as a mean (SD) based on a normal distribution, whereas categorical data were described as numbers and percentages. For comparing nominal categorical variables that are patient-related factors, Chi-squared test and Fisher Exact test (fisher test was utilized for 2*2 cells) were used. Fisher Exact test was used to calculate phenotype and genotype statistics. P-values are of or less than 0.05 were considered statistically significant. Statistical Package for the Social Sciences version (SPSS) 23.0 was used to do all calculations.

Results

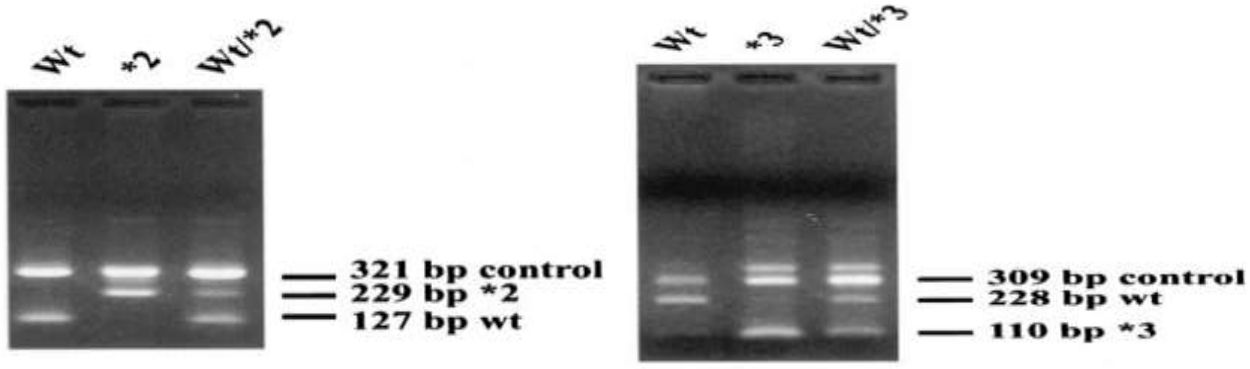
Characteristics of the patients

Sixty patients of 34 males and 26 females, with ages ranging from 38 to 96 years were involved in the study. The proportions of diabetes, smokers and hyperlipidemia were higher in the recurrent stroke group whereas the proportions of hypertension, ischemic heart diseases (IHDs), family history and clopidogrel drug brand are nearly equal in both groups. However ACE/ARBs, beta-blockers and statins usage percentages are higher in the non-recurrent group. Only the CYP2C19*2 was associated with the incidence of stroke recurrence (p=0.024), but not the CYP2C19*3 loss of function (p=1.000). The gel photographs of CYP2C19 polymorphisms are depicted in figure 1. Among the rest of variables**,** there was also a significant correlation between the usage of ACEs/ARBs and stroke recurrence (p=0.024).

TableError! No text of specified style in document.**1. Demographic and clinical characteristics of the study sample**

Variable	Total(n=60),%	Recurrent group(n=30),%	Non-recurrent group(n=30),%	P-value
Gender				0.602
Male	34(56.7)	16 (53.3)	18 (60.0)	
Female	26(43.3)	14 (46.7)	12 (40.0)	
Age, yrs.				0.100
<50	7(11.7)	2 (6.7)	5 (16.7)	
50-59	14(23.3)	7 (23.3)	7 (23.3)	
60-69	23(38.3)	9 (30.0)	14 (46.7)	
>70	16(26.7)	12 (40.0)	4 (13.3)	
Weight, kg (Mean ± SD)		77 ±11.305	81 ± 11.883	0.176
Hypertension	49(81.7)	24 (80.0)	25 (83.0)	0.739
Diabetes	23 (38.3)	13 (43.3)	10 (33.3)	0.426
IHD	7 (11.7)	3 (10.0)	4 (13.3)	1.000
Hyperlipidemia	39 (56.0)	18 (60.0)	21 (70.0)	0.417
Smoking	11 (18.3)	8 (26.7)	3 (10.0)	0.095
Current therapy				
ACEIs/ARBs	42 (70.0)	17 (56.7)	25 (83.3)	0.024
Beta-blockers	9 (15.0)	3 (10.0)	6 (20.0)	0.472
CCBs	33 (55.0)	17 (56.7)	16 (53.3)	0.795
Statins	43 (71.7)	19 (63.3)	24 (80.0)	0.152
Anti-DMs	22 (36.7)	12 (40.0)	10 (33.3)	0.592
Aspirin	8 (13.3)	5 (16.7)	3 (10.0)	0.706
Stroke family history	11 (18.3)	6 (20.0)	5 (16.7)	0.739
Clopidogrel drug brand	5 (8.3)	3 (10.0)	2 (6.7)	1.000
CYP2C19*2 LOF	18 (30.0)	13 (43.3)	5 (16.7)	0.024
CYP2C19*3 LOF	5 (8.3)	2 (6.7)	3 (10.0)	1.000

IHD = ischemic heart disease, ACEIs/ARBs =angiotensin-converting-enzymes-inhibitors, CCBs =calcium-channel-blockers, Anti-DMs = anti-diabetics, LOF = loss of function. *Significant difference using Fisher Exact test ≤ 0.05 level



A B

Figure1. Polymerase chain reaction and tetra ARMS pattern of (A):CYP2C19*2 (681 G>A) Polymorphism and (B):CYP2C19*3 (636 G>A) Polymorphism. ARMS=Amplification- Refractory -Mutation-System. PCR = Polymerase Chain Reaction. Wt. - Wild type, bp- base pair.

Genotype distribution

In the study population (n = 60), the frequency of CYP2C19 genotypes is summarized in table 2. More than half of the participants (61.7%) are homozygotes for the wild type allele (*1/*1); 26.7 % are heterozygotes for the CYP2C19*2 allele (1*/2*); 6.7 % are heterozygotes for the CYP2C19*3 allele (*1/*3); 3.3 % homozygotes for the (*2 / *2) ; 1.7 % are homozygotes for CYP2C19*3 (*3/*3) as shown in figure 2. In the sample population, the (*2/*3) genotype was not observed. Based on these frequencies of genotypes,

CYP2C19*1,*2,*3 allele frequencies were 75.83%, 16.7%, and 5%, respectively.

Table 2. Genotype distribution of *CYP2C19.*

CYP2C19	Genotype	Frequency	%
CYP2C19*1	(*1/*1)	37	61.7
CYP2C19*2	(*1/*2) (*2/*2)	16 2	26.7 3.3
CYP2C19*3	(*1/*3) (*3/*3)	4 1	6.7 1.7
Total		60	100

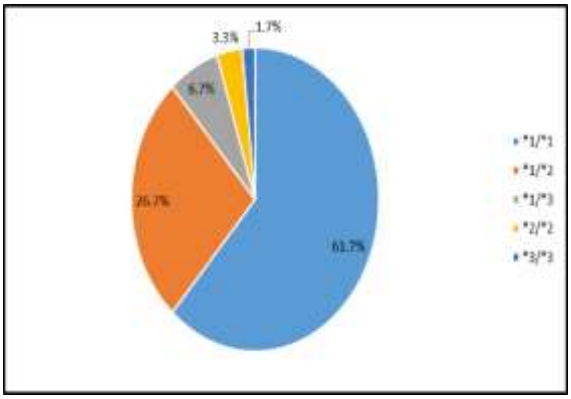


Figure2. Genotype distribution

CYP2C19 polymorphism and risk of stroke recurrence

Poor metabolizers (PMs) were found in two cases in the recurrent ischemic stroke group and one case in the non-recurrence group. Thirteen intermediate metabolizers (IMs) patients experienced a recurrent stroke, while seven patients had no recurrent stroke. The phenotypic distribution in both groups was not significant (p=0.176). The detailed data is portrayed in table 3.The distribution of CYP2C19*2 and CYP2C19*3 genotype in the two groups is shown in table 4, with the recurrent group accounting for the majority of the heterozygote instances [12] for the CYP2C19*2 allele. The statistical significance of this distribution was determined to be 0.039. There was one case with the (*3/*3) genotype and one case among four cases with the (1*/3*) genotype in the recurrent IS group. This distribution of CYP2C19*3 genotype and phenotype was not statistically significant (P=0.617).

Table 3. Genotype and phenotype association with recurrent ischemic stroke.

Phenotype	Genotype	Recurrent group(n=30),%	Non-recurrent group (n=30),%	P-value
Ems	GG	15(50)	22(73.3)	0.176
IMs	GA	13(43.3)	7(23.3)	
PMs	AA	2(6.7)	1(3.3)	

EMs = extensive metabolizers, IMs = intermediate metabolizers, PMs = poor metabolizers.

*Significant difference using Fisher Exact test <0.05 level.

Table4.CYP2C19*2 and *3 genotype and phenotype association with recurrent ischemicstroke.

Phenotype	Genotype	Recurrent group(n=30),%	Non-recurrent group (n=30),%	P-value
*CYP2C19*2*				
EMS	GG(*1/*1)	17(56.7)	25(83.3)	0.039
IMS	GA*(*1/*2)*	12(40)	4(13.3)	
PMs	AA(*2/*2)	1(3.3)	1(3.3)	
*CYP2C19*3*				
EMS	GG(*1/*1)	28(93.3)	27(90)	0.612
IMs	*GA(*1/*3)*	1(3.3)	3(10)	
PMs	*AA(*3/*3)*	1(3.3)	0(0)	

*Significant difference using Fisher Exact test <0.05 level.

Discussion

Genetic variants in CYP2C19, an enzyme essential for clopidogrel bio-activation, have been linked to lower clopidogrel antiplatelet efficacy and therefore to a higher risk of recurrence of ischemic stroke. The impact of the pharmacogenetics of the CYP2C19 polymorphisms CYP2C19*2 and CYP2C19*3 on the risk of stroke recurrence in ischemic stroke patients treated with clopidogrel for secondary prevention is reported in this study. Patients with CYP2C19*2 loss of function variant allele had a greater rate of stroke recurrence than those who did not have the mutant gene.

CYP2C19*1,*2,*3 allele frequencies were 75.83%, 16.7%, and 5%, respectively, while extensive metabolizers (EMs), intermediate metabolizers IMs, and poor metabolizers PMs allele frequencies were 61.7%, 20%, and 5%, respectively. The distribution of the CYP2C19*2 allele (16.7%) was found to be similar to an earlier Iraqi study which was done in 2015, on 221 people of Iraqi nationality and Arabic ethnicity who were not relatives with a CYP2C19*2 allele frequency of (15.2%) in addition to several studies included other Middle Eastern populations, such as Jordanians (16%), Lebanese (13.4%), and Palestinians (15.5%),[22- 25]. The CYP2C19*3 allele frequency in this study was (5%) which falls within the frequency range for Asian populations (3.36 % - 11.66%) which is reviewed in a meta-analysis study that included 78 original study articles [26]. The frequency of CYP2C19*3 allele in the sample population of this study was 5%, which differs from prior data in another study in 2018, Duhok city, in the Kurdistan region that was done by Mohammad and Al-Allawi in 2018 on 201 Iraqi patients on clopidogrel undergoing percutaneous coronary intervention which didn't document any case to carry this allele [27]. However, the number of patients carrying the heterozygote genotype (*1/*3) and homozygote genotype (*3/*3) in the both groups was small to compare, therefore we cannot depend considerably on them and we need a larger sample.

The data of the current study reveal that possessing the variant allele CYP2C19*2 (P=0.024) but not CYP2C19*3 (P=1.000) is associated with a potential risk for recurrent stroke and that the risk is linked with the IMs and PMs of CYP2C19*2 carriers (P=0.039). Similar findings were obtained in a study of patients treated with percutaneous coronary intervention (PCI) and dual antiplatelet therapy (DAPT), which indicated a significant link between main adverse cardiovascular events (MACE) and CYP2C19*2, but not CYP2C19*3 [25]. In contrast to the present study's findings, a study in Melbourne, Australia, found that CYP2C19*17 carriers had a higher risk of ischemia events (P=0.04), but CYP2C19*2 and CYP2C19*3 carriers had no significant association with the outcomes including ischemic events [28]. The explanation for

the difference between these conflicting results and the findings of the present study , regarding the relation of CYP2C19*2 and *3 polymorphisms with the recurrent risk of stroke, is may be due to the difference in the allelic frequency between populations since it is highest in Asian populations compared to others[26], as a result the low frequency of CYP2C19*2 and *3 in populations of Australia in the last study was not sufficient to make a strong correlation with the ischemic events. With regards to the significant relation of CYP2C19*17 with ischemic outcome, although this variant is associated with enzyme hyper-functioning leading to hemorrhagic events[29], this relation can be explained in two findings present in a review article made by Jiang et al, 2015, first one is that pharmacogenomics of antiplatelet intervention study (PAPI) founds that clopidogrel levels were similar in participants carrying the CYP2C19*17 allele and corresponding peers holding the CYP2C19*1 allele, suggesting that the CYP2C19*17 mutation has a minor impact on clopidogrel pharmacokinetics. Secondly, a recent pharmacogenetics study identified a linkage between the CYP2C19*17 allele and CYP2C19*4, an LOF mutation, which suggest that the high metabolic capacity of CYP2C19*17 carriers is altered if The CYP2C19*4B haplotype is also present in these individuals[10]. In conclusion, the ischemic outcomes may be resulted from another factor or mechanism particularly there was no association between CYP2C19*17 polymorphism and platelets activity as mentioned earlier.

Many additional non-genetic factors may contribute to an increased risk of stroke recurrence such as gender and race. Stroke prevalence is higher in black men than among other races[30]. All patients in that study were white without significant association with sex (P=0.602). Hypertension, ischemia, endothelial dysfunction, and pressure overload all promote local tissue ACE production, which can lead to long-term structural alterations such as myocardial and vascular remodeling. ACE inhibitors are thought to work by lowering blood pressure as a result of vasodilation and salt depletion as well as other benefits mediated by distinct vascular protective actions that lead to atherosclerosis regression or prevention[31]**.** However, the study findings revealed a significant relationship with ACEI/ARB medication therapy (P=0.024) and there was no significant association between recurrence risk and hypertension (P=0.739). These results were consistent with the findings of another prospective study in China 2019, done on 289 patients with ischemic stroke treated with clopidogrel for prevention where the

correlation of drug therapy during follow-up (ACEI/ARB) was significant with risk of stroke recurrence (P=0.006) and contrast with hypertension which was an independent risk factor of recurrence risk (P=0.04)[32], this can be due to that most of the hypertensive patients in the current study were of age below 60 years old, they had either hypertension alone or with one other risk factor, mostly hyperlipidemia without other comorbidities or they had controlled blood pressure. The majority of stroke prevention data in diabetic individuals comes from primary prevention rather than secondary prevention, Glycemic management has been proven to lower the risk of microvascular problems, but not stroke[30]. The correlation between stroke recurrence risk and diabetes in this study was not significant (P=0.426), this result was compatible with the other study results in China in patients undergoing stent plantation for cerebral artery stenosis [33]. In opposite, the correlation with diabetes was significant (P=0.009) in a study that was done in Spain on 209 TIA patients among and ischemic heart disease patients managed with stenting and dual antiplatelet follow-up for ischemic events[34]. Brand clopidogrel is approved for clinical use as hydrogen sulfate (bisulfate) salt whereas generic clopidogrel is designed with different salt formulations[35]. Different salt forms of any active component can vary markedly in physicochemical properties, such as solubility, hygroscopicity, stability and flowability, in addition to the presence of different levels of impurities between many copies of drug, can affect the biological activity of drug [20,21]. As a result, the efficacy of clopidogrel may vary with the use of brand and generic clopidogrel affecting the risk of stroke recurrence. However, the current study findings revealed that there is no difference in effect between patients using clopidogrel (P=1.000), similar to a retrospective study in USA, resulted that generic clopidogrel is as effective as the brand clopidogrel (P=0.77) for the inhibition of platelet aggregation[36], the same results also were found in other study in Ontario, Canada, on patients hospitalized for acute coronary syndrome including those had stroke or transient ischemic attack (TIA) taking branded and generic clopidogrel where there was no significant difference between clinical outcomes of death or re-hospitalization with the type of clopidogrel used (P=0.605) for ischemic stroke or TIA patients [37] .

Conclusion

1. This study provides certain evidence on the genetic effect of CYP2C19*2 on the risk of stroke recurrence in intermediate and poor metabolizers of clopidogrel drug in ischemic stroke patients since this variant can decrease the activity of CYP2C19 enzyme leading to decrease or even inhibit clopidogrel metabolism resulting in lowering the clopidogrel active metabolite level. As a result the antiplatelet action of clopidogrel is diminished.
2. This study affirmed that the ACE/ARBs drugs play a protective role in ischemic stroke patients since they are contributing to the prevention of ischemic stroke recurrence.

Recommendation

The CYP2C19 gene polymorphisms of patients were discovered to be effective in guiding therapeutic customized antiplatelet therapy. Modifying antiplatelet medication treatment based on CYP2C19 genotype such as increasing drug dosage, mixing drugs, or trying with new antiplatelet agents could be possible options to decrease clopidogrel resistance.

Limitations

This study has a number of drawbacks. Particularly, the study time length was insufficient in considering the recurrence of stroke, as well the limited sample size of the study.

References

1. Phipps MS, Cronin CA. Management of acute ischemic stroke. BMJ. 2020;368.
2. Cavallari LH, Momary KM. Pharmacogenetics in Cardiovascular Diseases. Pharmacogenomics. 2019. 133–179 p.
3. Catanese L, Tarsia J, Fisher M. Acute Ischemic Stroke Therapy Overview. Circ Res. 2017;120(3):541–58.
4. Oza R, Rundell K, Garcellano M. Recurrent Ischemic Stroke: Strategies for Prevention. Am Fam Physician. 2017;96(7):436–40.
5. Campbell BCV, De Silva DA, Macleod MR, Coutts SB, Schwamm LH, Davis SM, et al. Ischaemic stroke. Nat Rev Dis Prim [Internet]. 2019;5(1). Available from: http://dx.doi.org/10.1038/s41572-019-0118-8
6. Schneider S, Kornejeva A, Vibo R, Kõrv J. Risk Factors and Etiology of Young Ischemic Stroke Patients in Estonia. Stroke Res Treat. 2017;2017.
7. Hackam DG, Spence JD. Antiplatelet therapy in ischemic stroke and transient ischemic attack: An overview of major trials and meta-analyses. Stroke. 2019;50(3):773–8.
8. Borghol A, Onor I, Neuliep A, Zaki A, Andonie G, Zaki A, et al. Effectiveness of mono antiplatelet therapy vs dual antiplatelet therapy in ischemic stroke or transient ischemic attack—Special subgroup consideration for the African-American Population. Int J Clin Pract. 2020;74(7).
9. Hong KS, Lee SH, Kim EG, Cho KH, Chang D Il, Rha JH, et al. Recurrent Ischemic Lesions after Acute Atherothrombotic Stroke: Clopidogrel Plus Aspirin Versus Aspirin Alone. Stroke. 2016;47(9):2323–30.

10. Jiang XL, Samant S, Lesko LJ, Schmidt S. Clinical Pharmacokinetics and Pharmacodynamics of Clopidogrel. Clin Pharmacokinet. 2015;54(2):147–66.

11. Li C, Jia W, Li J, Li F, Ma J, Zhou L. Association with CYP2C19 polymorphisms and Clopidogrel in treatment of elderly stroke patients. BMC Neurol. 2021;21(1):1–7.

12. Golukhova EZ, Ryabinina MN, Bulaeva NI, Grigorian M V., Kubova MC, Serebruany VL. Clopidogrel response variability: Impact of genetic polymorphism and platelet biomarkers for predicting adverse outcomes poststenting. Am J Ther. 2015;22(3):222–30.

13. Jabir FA, Hoidy WH. Pharmacogenetics as Personalized Medicine: Association Investigation of SOD2 rs4880, CYP2C19 rs4244285, and FCGR2A rs1801274 Polymorphisms in a Breast Cancer Population in Iraqi Women. Clin Breast Cancer [Internet]. 2018;18(5):e863–8. Available from: http://dx.doi.org/10.1016/j.clbc.2018.01.009

14. Vidyanti AN, Chan L, Lin CL, Muo CH, Hsu CY, Chen YC, et al. Erratum: Aspirin better than clopidogrel on major adverse cardiovascular events reduction after ischemic stroke: A retrospective nationwide cohort study (PLoS ONE (2019) 14: 8 (e0221750) DOI: 10.1371/journal.pone.0221750). PLoS One. 2020;15(4):1–10.

15. Alhazzani AA, Munisamy M, Karunakaran G. Pharmacogenetics of CYP2C19 genetic polymorphism on clopidogrel response in patients with ischemic stroke from Saudi Arabia. Neurosciences. 2017;22(1):31–7.

16. Al-Talkani AF, Kathem SH. Prevalence of UGT1A1∗93 and ABCC5 polymorphisms in cancer patients receiving irinotecan-based chemotherapy at Al-Najaf Al-Ashraf. Iraqi J Pharm Sci. 2019;28(2):24–9.

17. Zanger UM, Schwab M. Pharmacology & Therapeutics Cytochrome P450 enzymes in drug metabolism : Regulation of gene expression , enzyme activities , and impact of genetic variation. Pharmacol Ther [Internet]. 2013;138(1):103–41. Available from: http://dx.doi.org/10.1016/j.pharmthera.2012.12.007

18. Wang Y, Zhao X, Lin J, Li H, Johnston SC, Lin Y, et al. Association between CYP2C19 loss-of-function allele status and efficacy of clopidogrel for risk reduction among patients with minor stroke or transient ischemic attack. JAMA - J Am Med Assoc. 2016;316(1):70–8.

19. Sacco RL, Adams R, Albers G, Alberts MJ, Benavente O, Furie K, et al. AHA / ASA Guideline Guidelines for Prevention of Stroke in Patients With Ischemic Stroke or Transient Ischemic Attack A Statement for Healthcare Professionals From the American Heart Association / American Stroke Association Council on Stroke Co-Sponsored by the Council on Cardiovascular Radiology. 2005.

20. Generic substitution: The use of medicinal products containing different salts and implications for safety and efficacy R.K. Verbeeck, I. Kanfer and R.B. Walker.

21. Gomez Y, Adams E, Hoogmartens J. Analysis of purity in 19 drug product tablets containing clopidogrel : 18 copies versus the original brand. 2004;34:341–8.

22. Sahib HA, Irhiem Mohammed B, Abdul-Majid BA. Genetic Polymorphism of Cyp2C19 in a Sample of Iraqi Population. Int J Pharm Biol Sci [Internet]. 2015;5(4):60. Available from: www.ijpbs.comorwww.ijpbsonline.com

23. Zalloum I, Hakooz N, Arafat T. Genetic polymorphism of CYP2C19 in a Jordanian population: Influence of allele frequencies of CYP2C19*1 and CYP2C19*2 on the pharmacokinetic profile of lansoprazole. Mol Biol Rep. 2011;39:4195–200.

24. Jureidini ID, Chamseddine N, Keleshian S, Naoufal R, Zahed L, Hakime N. Prevalence of CYP2C19 polymorphisms in the Lebanese population. Mol Biol Rep. 2011;38(8):5449–52.

25. Ayesh BM, Al-Astal IR, Yassin MM. The clinical effects of CYP2C19 *2 allele frequency on Palestinian patients receiving clopidogrel after percutaneous coronary intervention. Int J Clin Pharm [Internet]. 2019;41(1):96–103. Available from: https://doi.org/10.1007/s11096-018-00782-3

26. Céspedes-Garro C, Fricke-Galindo I, Naranjo MEG, Rodrigues-Soares F, Farinãs H, De Andrés F, et al. Worldwide interethnic variability and geographical distribution of CYP2C9 genotypes and phenotypes. Expert Opin Drug Metab Toxicol. 2015;11(12):1893–905.

27. Mohammad AM, Al-Allawi NAS. CYP2C19 genotype is an independent predictor of adverse cardiovascular outcome in iraqi patients on clopidogrel after percutaneous coronary intervention. J Cardiovasc Pharmacol. 2018;71(6):347–51.

28. Liu Z, Zhang C. Adverse events in cardiovascular disease patients taking clopidogrel : impact of CYP2C19 genotype polymorphisms. 2020;1–17.

29. Romano JG, Sacco RL. Prevention of Recurrent Ischemic Stroke. A Prim Stroke Prev Treat An Overv Based AHA/ASA Guidel. 2009;85–99.

30. Anderson C. Blood pressure-lowering for secondary prevention of stroke: ACE inhibition is the key. Stroke. 2003;34(5):1333–4.

31. Liu G, Yang S, Chen S. The correlation between recurrent risk and CYP2C19 gene polymorphisms in patients with ischemic stroke

treated with clopidogrel for prevention. Medicine (Baltimore). 2020;99(11):e19143.

32. Li YJ, Chen X, Tao LN, Hu XY, Wang XL, Song YQ. Association between CYP2C19 polymorphisms and clinical outcomes in patients undergoing stent procedure for cerebral artery stenosis. Sci Rep [Internet]. 2021;11(1):1–7. Available from: https://doi.org/10.1038/s41598-021-85580-0

33. González A, Moniche F, Cayuela A, García-Lozano JR, Torrecillas F, Escudero-Martínez I, et al. Effect of CYP2C19 polymorphisms on the platelet response to clopidogrel and influence on the effect of high versus standard dose clopidogrel in carotid artery stenting. Eur J Vasc Endovasc Surg [Internet]. 2016;51(2):175–86. Available from: http://dx.doi.org/10.1016/j.ejvs.2015.09.020

34. Serebruany VL, Hall TS, Atar D, Agewall S, Hyun Kim M, Geudelin B, et al. Mortality and adverse events with brand and generic clopidogrel in the US Food and Drug Administration Adverse Event Reporting System. Eur Hear J - Cardiovasc Pharmacother. 2019;5(4):210–5.

35. Westphal ES, Aladeen T, Vanini D, Rainka M, McCadden K, Gengo FM, et al. Generic Clopidogrel: Has Substitution for Brand Name Plavix® Been Effective? J Pharm Pract. 2021;

36. Ko DT, Krumholz HM, Tu J V., Austin PC, Stukel TA, Koh M, et al. Clinical outcomes of plavix and generic clopidogrel for patients hospitalized with an acute coronary syndrome. Circ Cardiovasc Qual Outcomes. 2018;11(3):1–9.