ORIGINAL PAPER

The Impact of Kinase Insert Domain (KDR) Gene Polymorphism rs2305948 on Clopidogrel Resistance in Iraqi Patients Undergoing Elective Percutaneous Coronary Intervention (PCI)

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ABSTRACT

Introduction: Clopidogrel, the first-choice antiplatelet agent for patient undergoing Percutaneous Coronary Intervention (PCI) along with Aspirin. Clopidogrel resistance is one of the major obstacles that cause MACE and failure of PCI. Kinase Insert Domain (KDR) gene responsible for VEGFR2 coding, the major receptor that translates VEGF ligand. The rs2305948 SNP in VEGFR2 gene has been documented to be involved atherogenesis and in CAD pathogenesis. **Aim**: To study the impact of KDR gene polymorphism rs2305948 on clopidogrel resistance in patients undergoing elective PCI. **Methods**: A case control study with 324 patients documented for elective PCI whom divided according to platelet aggregation level measured into (CR) with 111patients and (NCR) that consists of 213 patients. Serum lipids and VEGFR2 levels, BMI and platelet count were measured. Genotype for rs2305948 was done by PCR-RFLP. **Results:** Allele frequency and genotype results indicate a significant association with the pathogenesis of CR in all models in CR group compared to NCR group, a significant correlation for T allele with LDL, cholesterol and serum VEGFR2 in dominant and co-dominant models. RFLP-PCR results were documented by gene sequencing and results were compatible with HWE. **Conclusion:** rs2305948 SNP is associated with occurrences of CR and have an influence in the development of other metabolic changes.

Key words: Clopidogrel resistance, VEGFR2.

1. INTRODUCTION

Coronary Heart Disease CHD is a global leading cause of death result in 18 million deaths per year in 2016 with a cost of nearly $220 billion. Also, it is expected to increase to nearly 24 million deaths/year with an increase in cost by 100% (1). CHD occurs due to genetic and environmental risk factors such as DM, obesity, HT, smoking, alcohol consumption, sedentary life style. These factors lead to many defects on the cellular and molecular levels in vesicular inner wall that will end with formation of atherosclerotic lesion (2). The instability or rupture of atherosclerotic plaque is a superior triggering agent for the development of acute coronary syndrome (3). If the plaque becomes unstable and ruptures thrombosis, stroke, or myocardial infarction (MI) occurs. In a worldwide scale, from each 3 deaths,

there is a single death related to the rupture of a vulnerable plaque due to CHD (4). Despite numerous pharmacological interventions, PCI is still the management of choice for atherosclerotic plaque which produces ischemic heart disease (5).

1.1. Percutaneous Coronary Intervention (PCI)

PCI is the first-choice live saving maneuver for patients with STEMI attack, especially during the first two hours after documentation. It is used Ali A. Ahmed1, Najah R. Hadi1, Khalid I. Amber2 to restore myocardial coronary perfusions (6). The success of PCI is evaluated by clinical examination, angiography, preoperatively and after disappearance of the MACE. PCI performs rapid and more efficacious relief of ischemic sign and symptoms compared to noninvasive managements (7). The main problem that takes a

large concern regarding PCI procedure is the formation of a new thrombus (restenosis) that threats the life of the patient and may result in a new MI and death (8). The primary cause for thrombosis is platelet aggregation that will drive the formation of the fibrin mesh. So, the white thrombus is formed at first consisting mainly from platelet. Then, the red thrombus is later formed involving other components of blood (9). To inhibit this bad prognosis, the use of antiplatelet drugs (i.e. P2Y12 blockers as clopidogrel) is crucial. Also, the treatment guidelines recommend the use of dual anti platelet therapy (Aspirin and Clopidogrel), not only for patient whom has undergone PCI but also to patients with IHD (10). There is a large variability in the response of patient to standard doses of clopidogrel. These responses vary from bleeding in some patient (indicating a need to reduce the dose) or formation of a thrombus indicating that the patient doesn't't respond to the effect of clopidogrel as an inhibitor of platelet aggregation (clopidogrel resistance). In this case, there is a need to switch to another antiplatelet drug (i.e. Ticagrelor) (11). Some evaluating tests of clopidogrel that function as platelet aggregation inhibitor in human have been developed as Flow cytometry, Platelet function analyzer -100 system, Verify Now Test and Impedance aggregometry (12).

1.2.   Platelet: Giulio Bizzozero, the Italian researcher and Doctor, was the first who clarified the vital role for platelet in homeostasis and thrombosis in 1881 (13).

Platelet plays a major role in the complex process of homeostasis that utilizes many proteins and different biological interaction to produce its final outcome (formation of thrombi) (14).

1.3.   Process of coagulation and thrombus formation

Change in vascular blood flow hemodynamic, especially in coronary arteries, stimulates platelets adherence to the sub-endothelial proteins, specifically collagen and vWF, resulting in platelet activation, aggregation and lastly, the formation of platelet plug at the injury position (15). Normally, RBC travels in central lamina, and platelet circulation takes place at a place periphery of blood vessel, very close to the vascular endothelium. So, any stress factor (atrial fibrillation, infection, cancer, vessel injury) will cause platelet interaction with adhesive molecules at the damaged endothelial cells (16). The result of this is activation of (IIb/IIIa) receptor, that has the ability for fibrinogen binding and allows cross linking to adjacent platelet causing their aggregation and forming platelet thrombi (17). ADP, the main activator of platelet aggregation through binding to its P2Y1 and P2Y12 platelet surface receptors (18). ADP is released from both endothelial cells and RBC due to shear stress and damage of blood vessels. Additionally, ADP is released by activated platelets, from their dense granules. Regarding platelet aggregation process, it is proved now that P2Y12 and P2Y1 are present in human platelet and vascular endothelial cells (19).

1.4.   VEGFR2

Kinase insert domain receptor, other names are vas-

cular endothelial growth factor receptor 2 (VEGFR-2), CD309 (cluster of differentiation 309) and Flk1 (Fetal Liver Kinase 1). The gene responsible for encoding VEGFR2 called Kinase Insert Domain gene (KDR) (20). VEGFR2 mediates endothelial cell survival through direct activation of PI3K that will phosphorylate PKB/AKT. When AKT is activated it will inhibit apoptosis of endothelial cells via phosphorylation of caspase 9 and (BAD) which result in deactivation of their pro-apoptotic activity (21). VEGFR2 increases permeability of the vascular endothelium by activation of endothelial nitric oxide synthase through AKT induced phosphorylation of eNOS or via PLCγ dependent Ca2+ influx with the subsequent increase in production and level of nitric oxide (NO) (22).

1.4.1.   VEGFR2 and polymorphism

KDR gene is the gene that is responsible for the expression of VEGFR2. It is located on chromosome 4 on the long arm q at location 12. VEGFR2 consists of 1,356 amino acids and considered as the main receptor that translates the binding of VEGFA ligand to mediate cellular migration, proliferation and apoptosis resistance (23. The splicing of the KDR gene result in 679 AA amputated the extracellular domain of the receptor and produce a soluble sVEGFR2 which circulate in the blood stream and act as a selective inhibitor for lymphatic blood vessel growth (24).

1.5.   The +1192C>T (rs2305948) SNP in KDR gene

At the + 1192 position on the exon 7, the replacement of C allele by T nucleotide takes place. This causes abnormal production of amino acid with its consequence alteration of VEGFR2 function or what is called no synonymous substitution (25).

1.6.   Clopidogrel

Plavix® (clopidogrel bisulfate) belong to the second generation thienopyridine with irreversible inhibitory effect of its active metabolite to P2Y12 ADP receptors that mediates platelet activation and aggregation (26). Following GIT absorption, nearly 85% of the prodrug suffers inactivation by hydrolysis via carboxyl esterase enzyme and the only available clopidogrel percentage which is 15% will be activated by the hepatic cytochrome P450 system, mainly through CYP2C19 isoenzyme. Clopidogrel antiplatelet activity has been evaluated by many studies trying to explain the poor response in inhibiting platelet aggregation through the effect of gene single nucleotide polymorphism of the activating enzyme CYP2C19 (27). Additionally, there are other important contributing factors such as drug interaction (28), epigenetic factor, disease and demographic factors (29). Recently, there has been large concern and orientation in scientific researches towards genetic single nucleotide polymorphism as an effective contributor behind diminished activity of clopidogrel in inhibiting platelet aggregation (30). The recent studies clarify the significant association of genetic polymorphism in VEGFR2 gene receptor which is rs2305948 with atherosclerotic and coronary heart diseases (31, 32). So, it is possible that poor response to clopidogrel in Iraqi Arabic population may be attributed to this genetic variation (33).

2. AIM

The aim of the study is the impact of KDR gene polymorphism rs2305948 on clopidogrel resistance in patients undergoing elective PCI.

3. MATERIAL AND METHODS

This study is a case-control study, comprised of two groups (persons who have CR and NCR control group). The collection of specimens was done from September 2018 till February 2019.

2.1. Criteria for including patients in the study

Inclusion criteria:

The following criteria have been followed for patients whom included in the study:

a) Iraqi Arabic race,

b) Age is between 30 to 70 years old, and

c) Documented CHD and need for PCI.

Exclusion criteria:

a) HF and impairment in renal function,

b) Hepatic impairment,

c) Any recent hemorrhage,

d) Surgical intervention within 1 month before PCI,

e) Allergic reaction to clopidogrel, heparin, aspirin or for contrast media, and

f) Patients who have not responded to the change of PPI (omeprazole or esomeprazole) into pantoprazole.

Approval from the Ethical Committee (in the Faculty of medicine, Kufa University, was taken for the protocol of the study. Written plus oral approval for the study procedure had been taken from all patients who were enrolled in the study.

2.2. Preparation of patients for the study and PCI procedure

All enrolled patients have been examined and documented by specialist physicians for the criteria of inclusion and exclusion. They were selected from Al Najaf Center for cardiovascular surgery and cardiac catheterization in AL-Sadder Teaching Hospital in Al Najaf Al Ashraf governorate. The enrolled patients 324 were prepared at least one week before PCI procedure as follow:

a) Taking dual anti platelet therapy (75mg of clopidogrel+100 mg of aspirin) daily for at least one week before Pb)CI (34).

b) Admitting to hospital at least 24 hours before the PCI operation.

c) Shaving the pubic region.

d) Taking the loading dose of clopidogrel (600 mg) by taking 2 tablets every 2 hours in the last 12-14 hours before operation (35).

e) For all patients on omeprazole and esomeprazole, these PPI were replaced with pantoprazole before PCI (36).

2.3. Blood Sampling

Blood sample was taken from each patient from vein and Puncturing of the vein was done at the morning before subjecting to PCI procedure while the participants were fasting, (prior to PCI and after the 600 mg loading dose of clopidogrel). blood with a quantity of five milliliters of were taken from all then it was separated to three portions (37):

a) One ml was placed in heparinized tube specific for multiplate analyzer.

b) Two mills were placed in EDTA tube for DNA extraction and platelet count.

c) The last two ml were placed in a plane tube with normal room temperature for serum analysis.

After 10-15 minutes' coagulation occur for sample in the plane tube, then centrifuge samples at 2000 xg for fifteen minutes after separation, many portions of serum were warehouse at -20°C for phenotype analysis.

The heparinized blood sample for platelet responsiveness test doesn't required centrifugation, it used directly for applying the test.

2.4. Groups of the study

All enrolled patients were subjected to the ADP test by Multiplate® analyzer to detect the clopidogrel resistance. According to the result of test; the enrolled patients were divided into patient group that includes patients that the clopidogrel drug failed to inhibit aggregation of platelet in their blood (Clopidogrel Resistance group) and patients that respond to clopidogrel anti-platelet activity (control group or Non Clopidogrel Resistance group).

2.4.1. The patients group (CR group)

The first group is Clopidogrel Resistance group CR with 111 patients in which clopidogrel failed to inhibit platelet aggregation (74 males and 37 female) with age range (55.82±9.31) from the total 324 contributors in this study.

2.4.2. Control group (NCR group)

This second group is a non Clopidogrel Resistance group with 213 patients (159 male and a 54 female) with age range (57.67±7.99) was selected according to their responsiveness to clopidogrel therapy after clopidogrel loading dose according to results obtained for analysis of high on treatment Platelet Reactivity by using the Multiplate® analyzer by Roche Company.

Determination of Phenotype

Parameters of Anthropometry as height (meter), weight (kg) obtained by regularized methods. The estimation of BMI done; weight (kg), height (square meter), serum cholesterol, Low-density lipoprotein-cholesterol (LDL-C), serum triglycerides (TG), serum VEGFR2 and high-density lipoprotein- cholesterol (HDL-C) were measured.

2.5. Genotype measurements

Extraction of DNA was done by the employment of purification kit for DNA (Promega). PCR- (RFLP) technique was used for determination of Genotype regarding VEGFR2 SNP (rs2305948). The amplification was done using suitable primers and a HGMM mix kit (Promega). PCR Product was digested with restriction enzyme (Promega). Separation of digested products was done by the employment of 3% agarose gel.

Statistical analysis

SNP	Primers	Restric. Enz.
Rs2305948	F-ATCCTTGGTCACTCCGGGGTA R- TATGCTGTGCTTTGGAAGTTCAG	Rsal

Table 1. The sequence of rs2305948 SNP (38)

Continuous variables were illustrated by mean ±SD.

Student's t-test to express the means variance between CR and NCR. ANOVA test was applied for describing level rates of continuous parameters in genotypes through the SPSS v. 25 software (Chicago, IL SPSS Inc), non-numerical variables were used to express genotype distribution and allele frequency, Chi-squared test to assess the existence of differences of these variables. If p value was <0.05, then variations are considered significant.

Logical regression was obtained by SPSS software, to predict the relevance of allele frequencies and genotype to CR with various models of inheritance table 2.9. The rs2305948 SNP of KDR gene, it's C was the major allele; and T was the minor allele. The models of inheritance were summarized in table (3). Odds ratio (OR) is the expression for the results regarding dissection for allele frequencies and genotype allocation, P-value and confidence interval (CI- 95%). Outcome adjustment for sex, age, BMI, HT, DM and smoking, OR, CI 95% and P-values were re-estimated.

4. RESULTS

This research with 324 participants revealed CR group with a (111) patient with a 34.26% and a NCR group with 213 patients according to CR test done through Multiplate® analyzer device by Roche company.

5.1. Genotyping

By evaluating the ratio of A260/A280, concentration

Kind of cycle	temperature	time	Cycle number
Initial Denaturation	95 C	5 minutes	1
denaturation	95C	1 minute	
Annealing	58 C	1 minute	30
Extension	72 C	1 minute	1
The final Extension	72 C	10 minutes	1

Table 2. Schedule protocol of PCR for rs2305948

Parameter	NCR (control)	CR	P value
No. (male/female)	213 (159 /54)	111 (74/37)	0.13
Age	57.67 ± 7.99	55.82 ± 9.31	0.062
BMI	28.71 ± 4.23	29.42 ± 4.74	0.170
Cholesterol (mg/dl)	288.00 ± 9.64	290.67 ± 10.75	0.023
TG	240.79 ± 18.63	238.51 ± 19.45	0.299
VLDL	48.15 ± 3.67	47.70 ± 3.89	0.305
LDL	207.39 ± 11.53	210.43 ± 13.20	0.033
HDL	32.45 ± 3.19	32.54 ± 3.46	0.815
Platelet count (×103/ mm3)	240.41 ± 49.62	249.21± 67.33	0.182
KDR (pg/ml)	8075 ± 687	8098± 731	0.779
Diseased vessels	157	90	0.569
HT	192	102	0.90
DM	119	60	0.86
Smoker	103	62	0.46
CCBs	37	22	0.653
ACEI/ARBs	51	35	0.268
B-Blockers	131	70	0.894
Diuretics	64	38	0.580
PPI	33	26	0.150
Nitrates	104	59	0.672

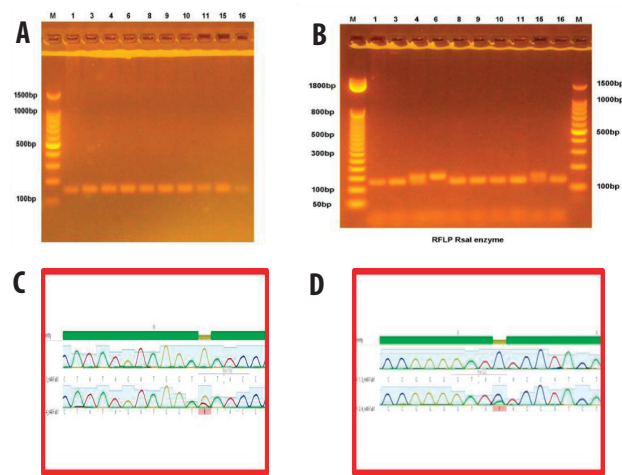Table 3. Baseline characteristics of study groups

Figure 1. (A) PCR product of rs2305948 with 151 bp. Ladder with 100 bp was used (B) Fragment of DNA for rs2305948, lanes number 1, 3,8,9,10,11 represent the wild genotype (CC), Lanes number 4 and 15 represents the heterozygous genotype (CT), Lane number 6 represents homozygous recessive genotype (TT). 50 bp and 100 bp ladders were used as ladders located on both side of the mold with the symbol (M), (C,D) genotype sequencing.

and purity were detected. The amplification product for VEGFR2 receptor gene polymorphism for rs2305948 (+1192C>T) is 151 bp. Results of amplification were analyzed and confirmed by electrophoresis on agarose gel as illustrated in figures 1 and 2 respectively.

5.2. RFLP analysis

The digestion of PCR product of rs2305948 SNP of

Genotype		No of bands	Size of bp
Wild type	CC	2	19, 132
Heterozygous	CT	3	19, 132, 151
Homozygous	TT	1	151

Table 4. Digestion results for rs2305948

VEGFR2 gene has been carried out by RsaI restriction enzyme. The agarose gel electrophoresis has been used to examine the digestion products. The outcomes demonstrated two (19, 132 bp) bands of wild type (CC), one (151 bp) band of homozygous (TT) and three (19, 132, 151 bp) bands of heterozygous (CT) genotypes as illustrated in Figures 1, 2 and Table 4. These results confirmed by the gene sequencing which was done for the rs2305948 as illustrated in Figure 3.

Estimation of Genotype and AlleleFrequencies

For the rs2305948 SNP, all genotyping and allele fre-

subject	χ2	P value
CR	0.232	0.630
NCR	1.634	0.201
Both	1.866	0.393

Table 5. Results of HWE analysis for rs2305948

quencies model shows a significant association with the occurrence of clopidogrel resistance before and after adjustment of the study parameters (BMI, DM, HT, Smoking, age and sex). For the (CT) heterozygous in the

rs2305948 (C/T)	Control N=213	CR N=111	Unadjusted OR.	Unadjusted (95%CI)	P-value	Adjusted OR.	Adjusted (95%CI)	P-value
			Co-dominant					
CC (Ref.)	173	66						
CT	36	38	2.76	1.61-4.73	0.000	3.13	1.76-5.57	0.000
TT	4	7	4.58	1.30-16.18	0.01	5.4	1.49-19.59	0.01
			Dominant					
TT+CT vs. CC	40	45	2.86	1.71-4.76	0.000	3.28	1.90-5.68	0.000
Recessive								
CC+CT (Ref.)	209	104						
TT	4	7	3.5	1.002-12.22	0.05	3.83	1.08-13.16	0.03
			Additive					
2(TT)+CT	44	52	3.09	1.89-5.06	0.0001			
MAF (T)	10.32	23.42	3.51	1.006-12.28	0.04			

Table 6. Genotype and allele frequency results of rs2305948 before and after adjustment of (BMI, sex, age, HT, DM, and Smoking)

Clinical characteristic	CC (66 patients)	CT (38 patients)	TT (7 patients)	P-value
LDL (mg/dl)	213.84 ±13.73	204.68± 9.70	209.42±15.31	0.002
VLDL (mg/dl)	47.31±3.25	48.50±4.84	47±3.41	0.28
HDL (mg/dl)	32.46±2.85	32.34±3.98	34.28±5.40	0.38
TG (mg/dl)	236.59±16.27	242.5±24.23	235±17.07	0.29
Cholesterol (mg/dl)	293.63±11.38	285.52±7.14	290.71±11.70	0.000
BMI (kg/m2)	28.64±4.71	28.84±4.09	29.95±4.55	0.762
Platelet count (×103/mm3)	259.84±26.64	266.21±22.29	243.71±8.26	0.073
VEGFR2 (pg/ml)	7682.72±135.47	8458.81±617.67	10067.14±153.07	0.000

Table 7. Biochemical characteristic of CR patients with genotype of rs2305948 SNP in the VEGFR2 gene under co dominant model

Clinical characteristic	CC (66 patients)	CT +TT (45 patients)	P-value
LDL (mg/dl)	213.84 ±13.73	205.42± 10.69	0.0008
VLDL (mg/dl)	47.31±3.25	48.22±4.65	0.227
HDL (mg/dl)	32.46±2.85	32.64±4.22	0.788
TG (mg/dl)	236.59±16.27	241.11±23.27	0.230
Cholesterol (mg/dl)	293.63±11.38	286.33±8.07	0.0003
BMI (kg/m2)	28.64±4.71	29.01±4.13	0.760
Platelet count (×103/mm3)	259.84±26.64	262.71±22.25	0.553
VEGFR2 (pg/ml)	7682.72±135.47	8709±819.46	0.000

Table 8. Biochemical characteristics of CR in patients with genotype of rs2305948 in VEGFR2 gene under dominant model.

co-dominant model that show a significant association with CR before and after adjustment of the study parameters (OR=2.76, CI 95%= 1.61-4.73 P=0.000) and (OR=3.13, CI95%=1.76-5.57, P=0.000) respectively. The minor homozygous genotype (TT) clarifies a significant association with CR before and after adjustment for parameters of the study (OR=4.58, CI 95%; 1.30-16.18, P=0.01) and (OR=5.4, CI 95%; 1.49-19.59, P=0.01) respectively. At the dominant pattern, TT+CT genotypes within CR persons were assure to be significantly associated with CR (OR=2.86, CI 95% =1.71- 4.76, P=0.000) at the unadjusted model and (OR=3.28, CI 95% =1.90- 5.68, P=0.000) at the adjusted model. The recessive model of genotype

CC+CT show an obvious significant correlation with CR group before (OR=3.5, CI 95%=1.002-12.22, P=0.05) and after adjustment (OR=3.83, CI 95%=1.08-13.16, P=0.03). At the additive pattern, 2(TT) + CT genotype was also significantly associated with CR group (OR= 3.09, CI 95% =1.89-5.06, P=0.0001). The minor allele (T) frequency was elucidate to be significantly increased in CR patients compared with control group (OR=3.51, CI 95%= 1.006- 12.28, P=0.04). Settlement for age, sex, BMI, DM, HT and smoking did not change the results as in Table 6.

5. DISCUSSION

Platelet aggregation test for clopidogrel responsiveness was done for the entire participant in the study. According to the results, it was found that 111 patients from the total 324 patients whom had been enrolled to PCI procedure expressed a resistance to the effect of clopidogrel with a percentage of 34.26% in our Iraqi Arabic enrolled population and this result is in coordinate with the study of (39) which states that the CR prevalence ranges from 5% to 44 % .

VEGFR2 gene rs2305948 SNP genotype and allele frequency

According to best of our knowledge, this study is the second study around the world and the first study in Iraq, the Middle East, Iran and Turkey (Iraq surrounding countries) that investigate clopidogrel resistance from Pharmacogenomic aspect through the existence of genetic polymorphism in the VEGFR2 gene, specifically; the rs2305948.

Outcome data of the rs2305948 SNP of KDR gene revealed a significant association with the presence of clopidogrel resistance in the Iraqi Arabic population enrolled in the study. Before adjustment of the study parameters (BMI, HT, DM, smoking, age and sex) and under the co-dominant model, patients whom carry the minor homozygous genotype (TT) with the heterozygous genotype (CT) have five folds and three folds increased in the risk to display the clopidogrel resistance respectively compared to the carriers of the wild type (CC) genotype. Additionally, under the dominant and recessive models

there was also a significant association with a two-fold and three-fold increase in the risk to develop CR respectively. This clarifies the strong influence of the rs2305948 SNP on CR. Carriers of the T allele (MAF) in the disease group (CR) are present with an increase in the risk to develop CR by more than three-folds as in Table 6. These results are in consistence with the study of (Zhang et al, 2016) (33). Adjustment of the BMI, HT, DM, smoking, age and sex doesn't't affect the outcome significantly as in table 6. This may be due to the fact that all the enrolled patients are with CHD; so, all of these factors have the same influence on both the clopidogrel resistant group and the non-clopidogrel resistant group giving the advantage for the genetic polymorphism to have prominent influence on this issue as illustrated in Table 6.

For the rs2305948, the serum level for VEGFR2, platelet count, serum lipids (LDL, VLDL, HDL, TG and cholesterol) and the BMI, were all tested with both the dominant model and the co-dominant model. The results as in tables 7 and 8 revealed the following:

a) Under the co-dominant model, the significant correlation of the MAF (T) with LDL and cholesterol concentration indicates a crucial role of these lipids on increasing the hazard to induce platelet aggregation and thrombus formation. Additionally, there was a significant link for the (T) allele with the increased serum level of VEGFR2. This indicates that the loss of function of VEGFR2 receptor is due to the strong impact of this SNP through alteration the response of VEGFR2.

b) Under the dominant model, the same results as in the co-dominant model, but the impact of T allele was expanded to include a significant correlation with platelet count in blood. This correlation introduces an additional evidence for the influence of this SNP on diminishing the effect of clopidogrel as an inhibitor for platelet aggregation. Indeed, the rs2305948 is located on the exon 7, which is considered as a coding region. So, this SNP will change the normal amino acid sequence with consequence protein change that alters the gene expression for VEGFR2 receptors and leads to their dysfunction (40). The genotype and allele frequency for the investigated SNP (rs2305948) are consistent with Hardy Weinberg Equilibrium (HWE) as illustrated in Table 5.

This indicates that the genotype allocation is constant in Iraqi Arabic population. So, any disturbance regarding the genotype and allele frequency will accounts for the involvement of this SNP with the pathogenesis of CR. Regarding the genetic power, it was 85.3% for rs2305948 which potentiate the research results as a level of 80% considered an accepted level for logical decision. The RFLP-PCR analysis results of the (rs2305948) was confirmed by gene sequencing which obviously clarifies the correct results of the analysis and the existence of this genetic polymorphism with its strong impact on the occurrence of CR.

6. CONCLUSION

The results of this study clarify that the rs2305948 SNP in the KDR gene have a strong influence on the presence of clopidogrel resistance in Iraqi Arabic population.

REFERENCES

1. Jun TJ, Kang SJ, Lee JG, Kweon J, Na W, Kang D, Kim D, Kim YH. Automated detection of vulnerable plaque in intravascular ultrasound images. Med Biol Eng Comput. 2019 Apr; 57(4): 863-876. doi: 10.1007/s11517-018-1925-x.

2. Bokma JP, Daily JA, Kovacs AH, Oechslin EN, Baumgartner H, Khairy P, et al. Learning strategies among adult CHD fellows. Cardiology in the young. 2019; 29(11): 1356-1360.

3. Bing R, Driessen RS, Knaapen P, Dweck MR. The clinical utility of hybrid imaging for the identification of vulnerable plaque and vulnerable patients. J Cardiovasc Comput Tomogr. Sep-Oct 2019; 13(5): 242-247. doi: 10.1016/j.jcct.2019.07.002.

4. Darcot E, Colotti R, Pellegrin M, Wilson A, Siegert S, Bouzourene K, et al. Towards Quantification of Inflammation in Atherosclerotic Plaque in the Clinic - Characterization and Optimization of Fluorine-19 MRI in Mice at 3 T. Sci Rep. 2019; 9(1): 17488.

5. Ueshima D, Yoshikawa S, Sasaoka T, Hatano Y, Kurihara Y, Maejima Y, Isobe M, Ashikaga T. Obesity paradox in the era of percutaneous coronary intervention with 2nd-generation drug-eluting stents: an analysis of a multicenter PCI registry. Heart Vessels. 2019 Feb; 34(2): 218-226. doi: 10.1007/s00380-018-1234-1.

6. Mehta SR, Wood DA, Storey RF, Mehran R, Bainey KR, Nguyen H. et al. Complete Revascularization with Multivesseles PCI for Myocardial Infarction. N Engl J Med. 2019 Oct 10; 381(15): 1411-1421. doi: 10.1056/NEJMoa1907775.

7. Rymer JA, O'Donnell CI, Plomondon ME, Hess PL, Donahue M, Hebert PL. et al. Same-day discharge among patients undergoing elective PCI: Insights from the VA CART Program. Am Heart J. 2019; 218: 75-83, Am Heart J. 2019 Dec; 218: 75-83. doi: 10.1016/j.ahj.2019.09.003.

8. Rymer JA, O'Donnell CI, Plomondon ME, Hess PL, Donahue M, Hebert PL. et al. Same-day discharge among patients undergoing elective PCI: Insights from the VA CART Program. Am Heart J. 2019 Dec; 218: 75-83. doi: 10.1016/j.ahj.2019.09.003.

9. De Luca G, Dirksen MT, Spaulding C, Kelbaek H, Schalij M, Thuesen L. et al. Time course, predictors and clinical implications of stent thrombosis following primary angioplasty. Insights from the DESERT cooperation. Thromb Haemost. 2013; 110(4): 826-833, Thromb Haemost. 2013 Oct; 110(4): 826-833. doi: 10.1160/TH13-02-0092.

10. Koupenova M, Kehrel BE, Corkrey HA, Freedman JE. Thrombosis and platelets: an update. Eur Heart J. 2017 Mar 14; 38(11): 785-791. doi: 10.1093/eurheartj/ehw550.

11. McLaughlin E, Leggett S, Kelsberg G, Safranek S. Dual Antiplatelet Therapy for Patients with Cardiovascular Disease. Am Fam Physician. 2019; 100(8): 463-464. doi: 10.3390/jcm7040074.

12. Bhatt DL, Pare G, Eikelboom JW, Simonsen KL, Emison ES, Fox KA. et al. The relationship between CYP2C19 polymorphisms and ischaemic and

bleeding outcomes in stable outpatients: the CHARISMA genetics study. Eur Heart J. 2012 Sep; 33(17): 2143-2150. doi: 10.1093/eurheartj/ehs059.

13. Paniccia R, Priora R, Liotta AA, Abbate R. Platelet function tests: a comparative review. Vasc Health Risk Manag. 2015 Feb 18; 11: 133-148, doi: 10.2147/VHRM.S44469.

14. Ribatti D, Crivellato E. Giulio Bizzozero and the discovery of platelets. Leuk Res. 2007 Oct; 31(10): 1339-1341. doi: 10.1016/j.leukres.2007.02.008.

15. Gaertner F, Massberg S. Blood coagulation in immunothrombosis-At the frontline of intravascular immunity. Semin Immunol. 2016 Dec; 28(6): 561-569. doi: 10.1016/j.smim.2016.10.010.

16. Jackson SP, Nesbitt WS, Westein E. Dynamics of platelet thrombus formation. J Thromb Haemost. 2009 Jul; 7 Suppl 1: 17-20. doi: 10.1111/j.1538-7836.2009.03401.x.

17. Gogia S, Neelamegham S. Role of fluid shear stress in regulating VWF structure, functions and related blood disorders. Biorheology. 2015; 52(5-6): 319-335. doi: 10.3233/BIR-15061.

18. Juratli MA, Menyaev YA, Sarimollaoglu M, Melerzanov AV, Nedosekin DA, Culp WC, et al. Noninvasive label-free detection of circulating white and red blood clots in deep vessels with a focused photo acoustic probe. Biomed Opt Express. 2018; 9(11): 5667-5677. doi: 10.1364/BOE.9.005667.

19. Kim JT, Park MS, Choi KH, Cho KH, Kim BJ, Park JM. et al. Comparative Effectiveness of Dual Antiplatelet Therapy With Aspirin and Clopidogrel Versus Aspirin Monotherapy in Acute, Nonminor Stroke: A Nationwide, Multicenter Registry-Based Study. Stroke. 2019; 50(11): 3147-3155. https://doi.org/10.1161/STROKEAHA.119.026044.

20. Hamilos M, Petousis S, Parthenakis F. Interaction between platelets and endothelium: from pathophysiology to new therapeutic options. Cardiovasc Diagn Ther. 2018; 8(5): 568-580. doi: 10.21037/cdt.2018.07.01

21. Nobusawa S, Stawski R, Kim YH, Nakazato Y, Ohgaki H. Amplification of the PDGFRA, KIT and KDR genes in glioblastoma: a population-based study. Neuropathology: official journal of the Japanese Society of Neuropathology. Neuropathology. 2011 Dec; 31(6): 583-588. doi: 10.1111/j.1440-1789.2011.01204.x.

22. Hou YQ, Yao Y, Bao YL, Song ZB, Yang C, Gao XL. et al. Juglanthraquinone C Induces Intracellular ROS Increase and Apoptosis by Activating the Akt/Foxo Signal Pathway in HCC Cells. Oxid Med Cell Longev. 2016; 2016: 4941623. doi: 10.1155/2016/4941623.

23. Zhang Y, Wang SJ, Han ZH, Li YQ, Xue JH, Gao DF, et al. PI3K/AKT signaling pathway plays a role in enhancement of eNOS activity by recombinant human angiotensin converting enzyme 2 in human umbilical vein endothelial cells. Int J Clin Exp Pathol. 2014; 7(11):8112-7, PMID: 25550859, PMCID: PMC4270588.

24. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. Nat Rev Mol Cell Biol. 2006 May; 7(5): 359-371. doi: 10.1038/nrm1911.

25. Albuquerque RJ, Hayashi T, Cho WG, Kleinman ME, Dridi S, Takeda A. et al. Alternatively spliced vascular endothelial growth factor receptor-2 is an essential endogenous inhibitor of lymphatic vessel growth. Nat Med. 2009 Sep; 15(9): 1023-1030. doi: 10.1038/nm.2018.

26. Brockington A, Wokke B, Nixon H, Hartley J, Shaw PJ. Screening of the transcriptional regulatory regions of vascular endothelial growth factor receptor 2 (VEGFR2) in amyotrophic lateral sclerosis. BMC Med Genet. 2007; 8: 23.

27. Kalra K, Franzese CJ, Gesheff MG, Lev EI, Pandya S, Bliden KP, et al.

28. Pharmacology of antiplatelet agents. Curr Atheroscler Rep. 2013 Dec; 15(12): 371. doi: 10.1007/s11883-013-0371-3.

28. Ali Z, Elewa H. The Effect of CYP2C19 and Nongenetic Factors on Clopidogrel Responsiveness in the MENA Region: A Systematic Review. Clin Appl Thromb Hemost, Jan-Dec 2019; 25: 1076029619875520. doi: 10.1177/1076029619875520.

29. Jiang XL, Samant S, Lesko LJ, Schmidt S. Clinical pharmacokinetics and pharmacodynamics of clopidogrel. Clin Pharmacokinet. 2015 Feb; 54(2): 147-166. doi: 10.1007/s40262-014-230-6.

30. Brown SA, Pereira N. Pharmacogenomic Impact of CYP2C19 Variation on Clopidogrel Therapy in Precision Cardiovascular Medicine. J Pers Med. 2018; 8(1). doi: 10.3390/jpm8010008.

31. Zhong WP, Wu H, Chen JY, Li XX, Lin HM, Zhang B. et al. Genome wide Association Study Identifies Novel Genetic Loci That Modify Antiplatelet Effects and Pharmacokinetics of Clopidogrel. Clin Pharmacol Ther. 2017; 101(6): 791-802.

32. Li L, Pan Y, Dai L, Liu B, Zhang D. Association of Genetic Polymorphisms on Vascular Endothelial Growth Factor and its Receptor Genes with Susceptibility to Coronary Heart Disease. Med Sci Monit. 2016 Jan 4; 22: 31-40. doi: 10.12659/msm.895163.

33. Liu D, Song J, Ji X, Liu Z, Cong M, Hu B. Association of Genetic Polymorphisms on VEGFA and VEGFR2 with Risk of Coronary Heart Disease. Medicine (Baltimore). 2016; 95(19): e3413.

34. Zhang LJ, Zhang YQ, Han X, Zhang ZT, Zhang ZQ. Association of VEGFR-2 Gene Polymorphisms with Clopidogrel Resistance in Patients with Coronary Heart Disease. Am J Ther, Nov/Dev 2016; 23(6): e1663-e1670. doi: 10.1097/MJT.0000000000000231.

35. Yu HR, Wei YY, Ma JG, Geng XY. Beneficial effects of combined administration of Clopidogrel and Aspirin on the levels of proinflammatory cytokines, cardiac function, and prognosis in ST-segment elevation myocardial infarction: A comparative study. Medicine (Baltimore). 2018; 97(45): e13010. doi: 10.1097/md.0000000000013010.

36. Koul S, Andell P, Martinsson A, Smith JG, Schersten F, Harnek J, et al. A pharmacodynamics comparison of 5 anti-platelet protocols in patients with ST-elevation myocardial infarction undergoing primary PCI. BMC Cardiovasc Disord. 2014; 14: 189.

37. Harvey A, Modak A, Dery U, Roy M, Rinfret S, Bertrand OF, et al. Changes in CYP2C19 enzyme activity evaluated by the [(13)C]-pantoprazole breath test after co-administration of clopidogrel and proton pump inhibitors following percutaneous coronary intervention and correlation to platelet reactivity. J Breath Res. 2016; 10(1): 017104. doi: 10.1088/1752-7155/10/1/017104.

38. Bolenius K, Lindkvist M, Brulin C, Grankvist K, Nilsson K, Soderberg J. Impact of a large-scale educational intervention program on venous blood specimen collection practices. BMC Health Serv Res. 2013; 13: 463.

39. Shahsavari S, Noormohammadi Z, Zare Karizi S. Association of kinase insert domain-containing receptor (KDR) gene polymorphism/ haplotypes with recurrent spontaneous abortion and genetic structure. Int J Reprod Biomed (Yazd). 2015; 13(12): 755-764.

40. Zhang J, Yang J, Chen Y, Mao Q, Li S, Xiong W. et al. Genetic Variants of VEGF (rs201963 and rs3025039) and KDR (rs7667298, rs2305948, and rs1870377) Are Associated with Glioma Risk in a Han Chinese Population: a Case-Control Study. Mol Neurobiol. 2016 May; 53(4): 2610-268. doi: 10.1007/s12035-015-9240-0.