

Study of Association between RAGE Gene Polymorphism rs2070600 (G82S) and Aspirin Resistance in Coronary Artery Disease Iraqi Patients with and without Type 2 Diabetes

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Abstract

Background: Despite Aspirin efficacy in cardiovascular disease, several patients on Aspirin therapy continue suffering thrombotic complications. Aspirin resistance phenomena is usually accompanied by potentially harmful consequences. The etiology is likely to be multifactorial. Several evidences suggested that genetic factors contribute significantly to the risk of aspirin resistance.

RAGE is a multiligand cell surface receptor. It is expressed in many cells including platelets. RAGE/ligand interaction has been evidenced to play a major role in oxidative stress mediated complications in coronary artery disease (CAD) and diabetes mellitus (DM). RAGE is implicated in platelet activation under pathological conditions. A functional polymorphism rs2070600 in the gene coding RAGE might modulate its receptor function. It is associated with enhanced ligand binding, leading to an enhanced receptor signaling and increased the ligand-stimulated generation of inflammatory mediators.

Objectives: The present study is aimed to evaluate the association between Receptor of Advanced Glycation End product (RAGE) – gene polymorphisms rs2070600 (G82S) and Aspirin resistance in coronary artery disease Iraqi patients with and without type 2 diabetes, and to detect the prevalence of Aspirin resistance in the studied population.

Patients and method: From February 2021 to October 2021, diabetic and non diabetic coronary artery disease (CAD) patients 225 (161 males, 64 females) already they were on aspirin 100 mg as prophylaxis were enrolled in a cross-sectional study, in addition to 130 (97 males,33 females) apparently healthy participants not taking Aspirin served as control group.

The response to Aspirin was evaluated by measurement the serum level of thromboxane B2 (TBX2), which is the more stable and measurable metabolite due activity of cyclooxygenase-1 (COX-1) enzyme that directly inhibited by Aspirin. Accordingly, the patients were divided into two groups: sensitive and resistant to aspirin.

Polymerase chain reaction amplification of the extracted deoxyribonucleic acid, and sequencing by Sanger method were used to identify the polymorphism of mostly related single nucleotide polymorphism (SNP) of RAGE (rs2070600).

Results: Prevalence of Aspirin resistance for all patients was 17.8%. For non diabetic CAD patients the prevalence was 16.1%, while for diabetic CAD patients the prevalence was relatively higher (19.6%). For rs2070600(G>A) frequency, there was no significant difference between resistant and sensitive groups (p value >0.05). However, a significantly higher serum levels of RAGE were detected in Aspirin- resistant groups compared with sensitive one in CAD patient; whether they were diabetic or non-diabetic, and the study showed a highly significant positive correlation between serum levels of RAGE and CRP.

Conclusion: The prevalence of Aspirin resistance among the studied Iraqi patients was relatively high and risky and could represent a challenge in considering Aspirin therapy for CAD patients by the specialists to be aware about such issue. However, our study didn't show significant association between Gly82Ser variants (rs2070600) with the risk of Aspirin resistance.

Keywords: Coronary Artery Disease; Aspirin Resistance; Type 2 Diabetes

INTRODUCTION

Coronary artery disease (CAD) is the leading non-communicable cause of cardiovascular mortality worldwide[1][2].

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Diabetes mellitus (DM) is a universal metabolic challenge, it is one of the most risk factor for coronary artery disease (CAD) and related complications[3]–[5]. It is well established that people with DM are presented with hyperreactive platelets and they are risky for thrombus formation[6]. Platelets play an important role in atherothrombosis, which is the major pathological alteration and primary cause of CVD[7][8].

Aspirin has been a corner stone and widely used for primary and secondary prevention of different cardiovascular diseases[9]–[11]. The therapeutic efficacy of aspirin in cardiovascular diseases is attributed to its ability to irreversibly acetylates platelet cyclooxygenase-1 (COX-1) leading to inhibition of thromboxane A2 synthesis by platelets hence, prevent platelet activation and aggregation[12]–[14].

Despite the wide use of aspirin indications, there is a significant number of patients do not respond to aspirin, who also are referred to as aspirin non-responders [15]. This phenomena also named as “Aspirin resistance” is a widely studied issue, also it refers to the concept of failure or reduced aspirin efficacy in preventing cardiovascular disease (clinical resistance) and improper inhibition of platelets and TXA2 synthesis (laboratory resistance)[16]–[19].

Advanced glycation end product receptor (RAGE, GenBank accession no. NC_000006) is localized on chromosome 6p21 and is a major controller of endogenous inflammatory effects [20] and reactive oxygen species[21].

In homeostasis, RAGE is usually expressed at low levels in adult, non-diseased tissues. In settings such as cardiovascular disease, diabetes, and inflammation, expression of RAGE is higher than that observed in control, non-diseased animal models or human subjects [22]. The RAGE gene encodes the advanced glycosylation end product receptor, belongs to the immunoglobulin superfamily of cell surface receptors[23]. Because of its special domain[24], it can bind multiple ligands[25] e.g. advanced glycation end products, β -sheets, S100, and HMGB1. This binding allows the receptors to activate oxidant stress sensitive nuclear factor- κ [26]. The receptor is expressed by many cell types [27][28], including platelets[29].

Many studies concluded that RAGE has crucial role in platelets hyperactivation and thrombus formation under pathological conditions[30]–[32]. Furthermore

RAGE gene expression is increased and implicated in many pathologic conditions including coronary artery disease and diabetic complications [33]–[35]. The relationship between RAGE gene polymorphism and vascular complications both in CAD and DM patients were extensively studied [36][37].

During the last decades, a growing number of clinical trials have studied the relationship of different genetic polymorphisms with aspirin resistance[17], [38]–[40].

RAGE (rs2070600) SNPs were widely studied and showed significant association with CAD diabetic complications

[41][42].

However, to our best knowledge there is no study evaluates the possible role of RAGE gene polymorphism in Aspirin resistance. We proposed that the RAGE genetic polymorphism may be associated with the risk Aspirin resistance. In order to test the hypothesis, we assessed the association of the RAGE rs2070600(G>A) genetic variants with the risk of Aspirin resistance in coronary artery disease Iraqi patient with and without diabetes.

Patients and method

After approval of our protocols by the Scientific and Ethical Committee in College of Pharmacy, University of Baghdad and Research and Development Committee/ Wasit Health Directorate/Ministry of Health in Iraq and in accordance with the contemporary Declaration of Helsinki, a cross sectional study was conducted in Al-Zahraa Teaching Hospital, Department of Cardiology, between February 2021 to October 2021. The study is a part of large study enrolled about 232 consecutive patients. Initially, we enrolled 232 diabetic and non diabetic stable coronary artery disease (CAD) patients. However, only 225 (161 male ;64 female). The excluded patients were: 2 patients due to non compliance; 5 patients due to non valid samples). The enrolled patients already were on daily administration of Aspirin 100 mg as prophylaxis without missing any dose. (For at least the last 7 days before enrollment)

Patients are eligible for the study if they were 18 years or older having stable CAD with and without diabetes, had been diagnosed with type 2 diabetes and were treated with oral hypoglycaemic agents and/or insulin. All patients were diagnosed and treated under specialist supervision.

CAD defined as at least one of the followings[43][44]:

1. $\geq 50\%$ stenosis in at least 1 coronary artery at cardiac catheterization.
2. percutaneous coronary intervention (≥ 6 months previously) or Previous myocardial infarction (≥ 6 months previously).
3. Coronary bypass surgery (≥ 12 months previously).
4. Abnormal exercise treadmill test (defined as at least 1 mm of horizontal or down sloping ST depression in at least 2 contiguous ECG leads) or an area of reversible ischemia on nuclear imaging.
5. Pharmacologic stress or stress echocardiography with subsequent revascularization.

The diagnosis of type 2 DM is made when one of the following criteria is met :HbA1c of $\geq 6.5\%$ and or Fasting blood glucose (FBG) (no caloric intake for at least 8 hours) of ≥ 126 mg/dL [45].

Whereas those patients were excluded if they were

Using of anticoagulants or any drugs known to affect platelet function (including clopidogrel and non-steroidal anti-inflammatory drugs, ticlopidine, dipyridamole, or warfarin). Or had been administration of heparin or low-molecular weight heparin within 24 hours before enrolment. Or had a family or personal bleeding disorder. Or, had haemoglobin <

8 g/dl. Or, those had acute myocardial infarction within the last 30 days. Or, any percutaneous coronary intervention within 30 days. Or, any stroke within 6 months. Or any concomitant surgery (valvular or maze procedure). Or had a major surgery a week before enrolment. Or renal insufficiency (serum creatinine >1.2 mg/dl). Or any Liver disorders. Or had platelets count <150×10⁹/l or >450×10⁹/l. Or, International normalized ratio (INR)>1.2. Or had any chronic inflammation. Or any haematological or neoplastic diseases. Or being a pregnant female.

All patients were informed of the aims and nature of the study and because there was no clinical intervention where the patients were already on their own management including Aspirin, therefore verbal consent was gained from participants.

Apparently healthy participants 130 (97 males,33 females) not taking Aspirin or other non-steroidal for at least 10 days had served as control group, none of control had history of any vascular event until the study time. The control group required mainly to determine the TBX2 normal reference range.

Serum TBX2 level and other biochemical tests

About 8 mL-blood sample was drawn from every participant by antecubital venepuncture and divided into; two 1ml EDTA tubes one for DNA extraction, and the other for haematological tests. The remaining sample were allowed to clot at room temperature in gel tubes, then centrifuged at 3000 r.p.m. for 10 minutes. The separated serum samples were separated into several 1 ml Eppendorf tubes for biochemical assays and stored at(-80°C) until the time of assay. Some biochemical tests were done immediately

Data collection

Demographic data (age, gender, BMI, disease duration and others) and detailed medical history were collected by interviewing the patient using specifically designed information chart (Table-1).

Assays of TBX2 were performed altogether at the same time in thawed samples using commercially available ELIZA kits (SHANGHAI YEHUA Biological Technology Co., Ltd. CHINA), based on the protocol provided by the test manufacturer[46].

Criteria for definition of aspirin resistance

Several methods have been used to detect Aspirin resistance[47], however it was suggested that measuring the level of TXA2 derivatives may be a valuable non-invasive marker of TXA2 metabolism and for assessing the effectiveness of specific drugs[48].

The measurement of serum thromboxane B2, a stable metabolic product of TXA2, is the only test that measures the effect of aspirin on platelet COX-1 activity[49].

Therefore, the serum TxB2 test is considered the most accurate and appropriate method for assessing the pharmacological effects of aspirin and it also the most stable and reproducible test for determining the response to aspirin[49].

Since the serum TBX2 level can be obtained by different analytical methods is highly variable[50][51]. So we determined serum TBX2 reference interval specific for our study from an age, gender, BMI and ethnicity (all are Iraqis) matched apparently healthy control group[52]. Several previous studies used reference range obtained from healthy volunteers, although the methods for determination of aspirin resistance were different [53]–[55].

The reference range of our study was (407.82-671.47) pg/ml.

The response to Aspirin is evaluated by method of measurement the serum level of thromboxane B2(TBX2) of patients[56],and comparing with reference range obtained by control group. Therefore patient with serum TBX2 suppression by >97% of the lower limit of reference range(or < 12.23 pg/ml) was regarded as sensitive to Aspirin otherwise he is resistant to aspirin[57][58].

Accordingly, the patients were divided into two groups: sensitive and resistant to aspirin.

As a practical matter and from statistical view, it is best to design experiment so that sample sizes are equal [59]–[61]. So, an equal number of patients from sensitive group randomly selected to be compared with the obtained resistant group and to complete the next required analysis like PCR and DNA sequencing.

It is well documented that variability in patient compliance has a major effect on determination of response to aspirin regardless of the method used [62]. Therefore to ensure patient compliance and to avoid the variability in pharmacologic effect (response) of aspirin due to pharmacokinetic properties such as variability in absorption and hence bioavailability using different aspirin formulations [63], we measured the serum aspirin level in all enrolled patients [55], in addition to patient interview and asking in detail for their adherence with aspirin.

Table 1. Sociodemographic, clinical and biochemical characteristics of the participants

<i>Variable</i>	<i>Patients n=225</i>	<i>Control n=130</i>	<i>P value</i>
Gender ^d :Males	161(71.6%)	93(71.5%)	0.997 ^a
n (%) :Females	64(28.4%)	37(28.5%)	
Age (years) ^c	56.85±8.11	58.16±8.9	0.158 ^b
BMI (kg/m ²) ^c	27.94±1.72	27.76±2	0.4 ^b
Normal weight ^d	12(5.3%)	11(8.5%)	0.49 ^a
Over weight ^d	185(82.2%)	102(78.5%)	
Obese ^d	28(12.4%)	17(13.1%)	
Diabetes type 2 ^d	107(47.6%)	0(0%)	-
CAD ^d	118(52.4%)	0(0%)	-
Patients resistant to Aspirin ^d	40(17.8%)	-	-
Troponin TEST	-ve	-ve	-
INR ^c	0.97±0.064	0.98±0.063	0.48 ^b
PLAT. COUNT (×10 ⁹ /l) ^c	250.19±34.17	251.15±37.16	0.806 ^b
PCV (%) ^c	42.99±2.39	42.97±2.32	0.939 ^b
HGB (g/dl) ^c	14.33±0.79	14.32±0.77	0.98 ^b
RBCs (10 ³ /μl) ^c	5.41±0.45	5.4±0.43	0.944 ^b
WBCs(cell/μl) ^c	6211.73±217.18	6179.78±202.39	0.172 ^b
Scr (mg/dl) ^c	1.08±0.04	1.08±0.04	0.331 ^b
B urea (mg/dl) ^c	30.14±4.68	30.24±4.92	0.849 ^b
CHOL (mg/dl) ^c	187.69±29.97	182.79±32.18	0.15 ^b
TG (mg/dl) ^c	156.13±34.59	156.35±31.02	0.951 ^b
HDL (mg/dl) ^c	44.43±4.96	43.64±5.25	0.159 ^b
LDL (mg/dl) ^c	111.89±25.12	107.89±27.86	0.165 ^b
VLDL (mg/dl) ^c	31.22±6.9	31.27±6.2	0.942 ^b

CAD: coronary artery disease; DM: diabetes; BMI: body mass index; INR: international normalised ratio; PCV: packed cell volume; HGB=Heamoglobin ;CHOL: cholesterol; TG: Triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein; c: mean ± standard deviation; d:number(percent total).

**: highly Significant difference between the groups (significance P <0.01);

The final number of patients enrolled into the study was 225, where 118(52.4%) of them were non diabetic CAD patients, and 107(47.6%) were diabetic CAD patients.161(71>6%)

male and 64(28.4%) were female. The age was (56.85±8.11) years. Their BMI was (27.94±1.72) Kg/m² .40 of the total patients were resistant to Aspirin(table 1).

Table 2. The sequences of the primers, annealing temperature, product size (bp)

<i>Primer Name</i>	<i>Seq.</i>	<i>Annealing Temp (°C)</i>	<i>Product size (bp)</i>
rs2070600-F	5'-CTGCTTCTTCCACTACCTTAT -3'	63	706
rs2070600-R	5'-CCTCTACCATGGTGCTATCT -3'		

Primer optimization

To examine the optimum annealing temperature of primer, the DNA template was amplified with the same primer pair, (Forward) (Reverse), at annealing temperatures of 55, 58, 60, 63 and 65°C. PCR amplifications were performed with 20µl volumes containing 10µl GoTaq Green Master Mix (2X); 1µl for each primer (10pmol); 6µl nuclease free water and 2µl of template DNA.

PCR cycling was performed with PCR Express (Thermal Cycler, BioRad, USA) with the following temperature program: denatured at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 30 sec; annealing at 55, 58, 60, 63 or 65°C for 30 sec; and extension at 72°C for 30 sec. A final extension incubation of 7 min at 72°C was included, followed by a 10 min incubation at 4°C to stop the reactions.

Statistical analysis

Statistical analysis was performed using the IBM SPSS for Windows version 26.0 software (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± SD. Categorical variables such gender, Allele and genotypes were presented in number and percentage. Kolmogorov Smirnov test was used to assess the normality of the variables of the

results.

The unpaired t-test was used for normally distributed data to determine a significant difference in demographic characteristics and parameters between the sensitive and resistant groups. The chi-square test was used to test group differences of proportions. Furthermore, multiple variable binary logistic regression was used to detect the independent risk factors. $P < 0.05$ indicated a significant difference. Binary logistic regression analysis was used to predict the likelihood that a patient falls into which one of the two groups of a dichotomous dependent variable (aspirin resistance or aspirin sensitive) based on the independent variables that were continuous, and categorical. A reference interval is a range of reference values obtained in healthy individuals of the same or similar characteristics[64]. We used the nonparametric method that based on the determination of the 2.5 and 97.5 percentiles following sorting of the data, it has been used for TBX2 reference interval determination from apparently healthy volunteers[65][66].

RESULTS

Total Aspirin resistance in our study population was (18%), as it had been noted in 40 of 225 patients.

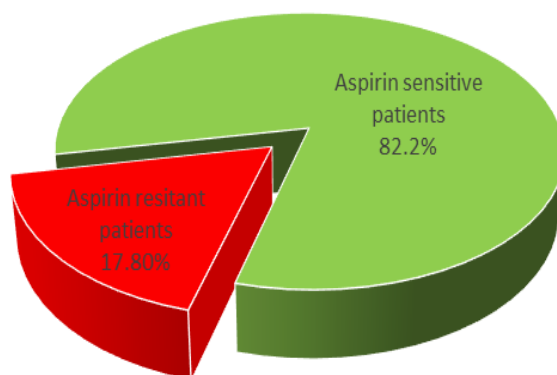


Fig. 1. The prevalence of Aspirin resistance Among enrolled patients

The frequency of aspirin resistance in CAD patient without DM was (16.1%), as compared with CAD patients with DM showed a higher frequency of Aspirin resistance (19.6%) in but no statistically significant difference was noted.

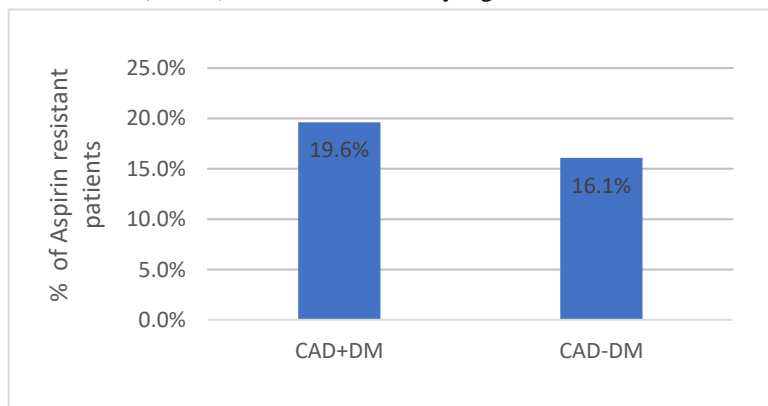


Fig. 2. The prevalence of Aspirin resistance among CAD patients with and without DM patients

Table -1 shows the base line and clinical characteristic of the studied patients and control. There were no significant differences between patients and controls regarding the

gender, the percentage of smokers, BMI, age, INR, haematologic parameters, serum creatinine, blood urea, and lipid profile($p>0.05$).

Table 3. Sociodemographic, clinical and biochemical characteristics of studied patients

Variable	Diabetic CAD patients			Non diabetic CAD patients		
	Resistant n=21	Sensitive n=20	P value	Resistant n=19	Sensitive n=20	P value
Gender n (%)	Male	14(66.7%)	0.658 ^a	12(63.2%)	14(70%)	0.651 ^a
	Female	7(33.3)		7(36.8%)	6(30%)	
Age (years)	54.71±7.79	56.4±6.57	0.46 ^b	56.05±7.66	57.35±8.9	0.629 ^b
BMI (kg/m ²)	28.19±1.71	27.4±1.94	0.169 ^b	28.38±0.99	27.95±1.57	0.309 ^b
Platelets count (×10 ⁹ /l)	244.1±37.81	243.4±34.15	0.951 ^b	253.37±28.18	258.05±34.33	0.645 ^b
PCV (%)	43.34±2.26	41.83±1.61	0.019 ^b	42.21±1.69	43.33±2.62	0.123 ^b
NLR	2.3±0.16	2.32±0.2	0.705 ^b	2.25±0.16	2.19±0.15	0.247 ^b
CRP (mg/l)	3.5±0.6	3.14±0.52	0.046 ^{*b}	3.29±0.47	2.95±0.41	0.021 ^{*b}
CHOL (mg/dl)	187.86±27.6	180.85±31.89	0.456 ^b	197.01±31.07	193.92±18.36	0.71 ^b
TG (mg/dl)	160.65±33.9	152.36±26.55	0.39 ^b	169.69±30.42	171.87±29.33	0.821 ^b
HDL (mg/dl)	42.37±5.68	45.81±5.3	0.051 ^b	43.46±3.79	43.76±5.43	0.842 ^b
LDL (mg/dl)	115.4±22.82	103.26±24.69	0.11 ^b	119.77±28.12	115.55±16.08	0.572 ^b
VLDL (mg/dl)	31.7±6.64	30.47±5.31	0.52 ^b	33.94±6.08	34.27±5.65	0.859 ^b
TBX ₂ (pg/ml)	527.01±83.31	8.33±1.99	0.00 ^{**b}	520.38±58.89	8.51±2.15	0.00 ^{**b}
ASP(µg/ml)	64.25±6.18	66.22±6.25	0.319 ^b	65.03±5.03	64.18±4.55	0.585 ^b
RAGE (ng/ml)	3.48±0.88	2.68±0.69	0.003 ^{**b}	3.2±0.92	2.46±0.66	0.006 ^{**b}

SD: Standard deviation; CAD: coronary artery disease; BMI: body mass index; INR: international normalised ratio; PCV: packed cell volume; NLR: neutrophil-lymphocyte ratio; HbA1c: glycated haemoglobin; CRP: C-reactive protein; CHOL: cholesterol; TG: Triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein; TBX₂: thromboxane B2 serum level;

ASP: Aspirin serum level; RAGE: advanced glycation end product receptor serum level;

*: Significant difference between the groups (significance $P < 0.05$);

** : highly Significant difference between the groups (significance $P < 0.01$);

***: very highly Significant difference between the groups (significance $P < 0.001$);

a: Chi-square test; b: Independent 2 sample t-test.

Table 3 describes the sociodemographic, clinical and biochemical characteristics of resistant and sensitive groups for both diabetic and non diabetic CAD patients.

There were no significant differences between Aspirin sensitive and resistant groups regarding to; gender, BMI, age,

INR, haematologic parameters, serum creatinine, blood urea, and lipid profile($p>0.05$). However, CRP, TBX₂ and RAGE serum concentrations were significantly higher in resistant subjects compared to sensitive subjects in both diabetic and non diabetic CAD patients ($p<0.05$).

Table 4. Genotype & allele frequency of RAGE (rs2070600G>A) polymorphisms Among Aspirin-resistant and sensitive groups (80)

polymorphism		<i>Diabetic CAD patients</i>			<i>Non diabetic CAD patients</i>		
		<i>Sensitiv e (n=20)</i>	<i>Resistanc e (n=21)</i>	<i>P value</i>	<i>Sensitiv e (n=20)</i>	<i>Resistanc e (n=19)</i>	<i>P value</i>
rs2070600(G>A)	Genotype						
	GG	18(90%)	20(95%)	0.52 0 ^a	19(95%)	19(100%)	0.32 ^a
	GA	2(10%)	1(4.8%)		1(5%)	0(0%)	
	Allele						
	G	38(97.4%)	41(97.6%)	0.52 8 ^a	39(97.5%)	38(100%)	0.32 ^a
	A	2(2.6%)	1(2.4%)		1(2.5%)	0(0%)	

a: Chi-square test.

Table 4 summarizes the genotype distributions and allele frequencies of RAGE polymorphisms in sensitive and resistant patients.

There were no significant differences of genotype distributions and alleles frequencies of rs2070600(G>A) between Aspirin sensitive and resistant subjects in both CAD with DM and without DM patients (all p>0.05).

Spearman correlation analysis for the incidence rate of Aspirin resistance with the risky variables explained by table 5. Only serum level of RAGE, CRP showed significant positive correlations (r: 0.492; 0.381 respectively) with the risk of Aspirin resistance (P<0.01). While there were no significant correlations regarding other variables (all P values >0.05), even with the frequency of RAGE rs2070600 (G>A) was non significant (P value; 0.311).

Table 5. Spearman’s correlations of Aspirin resistance with the studied risky variables of the patients.

<i>Parameter</i>	<i>Correlation Coefficient(r)</i>	<i>P value</i>
BMI	0.185	0.1
CRP	0.381	0.00**
CHOL	0.081	0.474
TG	0.082	0.468
HDL	-0.134	0.235
LDL	0.15	0.183
VLDL	0.057	0.617
RAGE	0.492	0.00**
rs2070600(G>A)	-0.115	0.311

** : highly Significant correlation (significance P <0.01)

To study the possible correlations between serum RAGE level and other studied risk variables for all patients, Pearson’s correlation was applied (table 6).

There were highly significant positive correlations between serum RAGE serum level with both ; BMI and CRP (P value<0.01). Also significant positive correlation with CHOL, TG, LDL, and VLDL (P value<0.05).

Table 6. Pearson's correlations of serum RAGE with the studied risk variables of the patients

<i>Parameter</i>	<i>Correlation Coefficient(r)</i>	<i>P value</i>
BMI	0.44	0.00**
NLR	0.087	0.442
CRP	0.567	0.00**
CHOL	0.246	0.028*
TG	0.279	0.012*
HDL	-0.190	0.091
LDL	0.225	0.044*
VLDL	0.284	0.011*

** : highly Significant correlation (significance P <0.01)
 * : Significant correlation (significance P <0.05)

DISCUSSION

Despite the wide use of Aspirin as an effective antiplatelet agent to prevent thrombi formation [67], there were a lot of patients do not respond to Aspirin, who also are referred to as aspirin non-responders and the phenomena called Aspirin resistance[15]. Prevalence of Aspirin resistance in patients with cardiovascular diseases is significantly variable (5% - 65%).

A few studies have been carried in Iraq, Ali T. Khlaif et al, found that the aspirin resistance prevalence was ranging between 1.8%-7.5%[68], in the North the result was 14%[69].

Our study has found out that total Aspirin resistance prevalence was 17.8%, for non diabetic CAD patients was 16.1%, while for diabetic CAD patients the prevalence was relatively higher (19.6%), (figure 2 and 3) however it stills non significant (P value >0.05).

A study from India by Chadha et al. [70]subdivided the decreased Aspirin activity into; poor response which 39% and non-response which was only 2%. In Pakistan similar study design found that Aspirin resistance was 12%[71]. MP Dorsch et al.[72]from North Carolina found that Aspirin resistance in CAD patients without myocardial infarction was 12%. These variabilities may be affected mainly by the method used for testing of Aspirin resistance [73]–[76].

Recent studies proposed several complex and multifactorial mechanisms of Aspirin resistance for example; obesity, dyslipidaemia, genetic also play a crucial role in aspirin resistance, and oxidative stress may have critical task in risk of Aspirin resistance, also patient adherence may be another mechanism[77][78].

Effect of obesity on aspirin response had been studied [76]. A recent study demonstrated that BMI was associated with reduced pharmacodynamic response to aspirin in patients with CAD[79]. It has predicted that 58% of world population are risky to be overweight or obese by the year

2030[80][81]. Thus, understanding the effect of obesity on aspirin resistance is essential.

Some mechanisms could explain the possible correlation between increased body weight and risk of Aspirin resistance. First; although aspirin is rapidly absorbed from the upper gastrointestinal tract, obese patients may have a different drug disposition due to higher volume of distribution [82]. Second; due to higher inactivation rate after entering the portal circulation through increased activity of carboxylesterases associated with obesity[83]. Other reason; it is documented that higher BMI and visceral obesity is associated with chronic inflammation, higher platelet volume, adipokines or insulin resistance all these could be associated with increased platelet activation and risk of predisposition to thrombosis[84].

Generally the obesity prevalence in Iraqi population is significant[85][86]and requires serious consideration by health policymakers and public health specialists to plan an effective and preventive provisions to avoid serious health consequences.

According to WHO definition of obesity[87], most of our participants even the control subjects were overweight and obese. Small percent of them had normal body weight (table 2). Although there was no significant correlation between BMI and risk Aspirin resistant, there was significant positive correlation between serum RAGE level and BMI (table 6).

Our results was contradictory with similar studies that showed significant correlations between increased body weight and tendency to be resistant to Aspirin [16]. On other hand our study was consistent other studies[70].

Risk of Aspirin resistance has been found to be associated with hyperlipidaemia, the later in turn is accompanied by an inflammatory and prothrombotic state where oxidised LDL(oxLDL) induces elevated sCD40L levels in hypercholesterolaemia[88]. Previous studies had proved that titres of sCD40L are positively correlated with platelet

activation markers of such as TXB2[89]. However our study showed no significant correlation between risk of Aspirin resistance and lipid profile, similar to some results [90], [91] and different from other studies[70]. In the same time our study showed a significant positive correlation between lipid profile and the RAGE serum level (table 6).

The above variations in results may be related to ethnicity, method used for testing platelet function and different definitions used for Aspirin resistance[92]–[95].

One of the most extensively studied biomarker of inflammation in cardiovascular disease is serum C-reactive protein (CRP) [96]–[98]. It is mainly produced by the liver under the stimulation of proinflammatory cytokines, including IL-6 and TNF- α [99].

Many studies showed a close correlation between abnormal platelet activity and oxidative stress in CVD and diabetes complications [100][101]. The relationship between oxidative stress and thromboxane(TX) biosynthesis was previously demonstrated in several clinical settings characterized by enhanced risk for atherothrombosis[102].

Oxidative stress is considered to be a stimulator for the formation of thromboxaneA2 from arachidonic acid by nonenzymatic or enzymatic pathways(e.g., via lipoxygenase) not blocked by aspirin resulting in aspirin resistant platelet aggregation[103]. This confirms a potential relationship between oxidative stress and aspirin resistance[104].

In our study, low response to aspirin in our patients had a significant correlation with high level of oxidative stress reflected by elevated serum values of CRP in CAD patients both with and without diabetes.

RAGE is a multiligand member of the immunoglobulin superfamily. It is expressed by many cell types including platelets[29].

Our study revealed a significantly higher serum level of RAGE in resistant groups compared with sensitive one in CAD patient whether they were diabetic or non-diabetic (table 3). The study showed a highly significant positive correlation between RAGE serum level and CRP (table 6).

RAGE activation by its ligands was found to increase classical acute phase reactants (e.g. C-reactive protein)[105][106]. ROS then activates NF-kB which in turn results in transcriptional activation of variety of inflammatory genes such as TNF- α , TNF- β , IL-1, IL-6, IL-8, and interferon gamma[107]–[110]. NF-kB induces gene for NADPH oxidase in polymorphonuclear leukocytes[111] which would generate further ROS.

Therefore, we can conclude that, the oxidative stress may be the connection between the RAGE activation and risk of Aspirin resistance.

The use of genetic data for establishing of new therapies requires the recognition of causative genetic variants and clarification of the molecular mechanisms by which they predispose to CAD[112].

RAGE gene is one of the most likely candidate genes that account for genetic predisposition to CAD. When RAGE activated it can provoke wide range of signalling pathways that trigger inflammation, atherogenesis and vasoconstriction leading to coronary dysfunction, atherosclerosis and thrombosis[113]. RAGE gene might be a powerful negative predictor of coronary artery disease[114].

RAGE (rs2070600G>A) also called (Gly82Ser) or (G82S), was widely studied and showed significant association with CAD diabetic complications [41][42]. The Gly82Ser polymorphism is located in exon 3 of the RAGE gene[42].

The 82G/S (82Gly \rightarrow Ser) occurs in the ligand-binding V-domain of RAGE and therefore has attracted great attention[115]. It has essential functional modulations, because it is associated with enhanced ligand binding, leading to an enhanced receptor signaling and increased the ligand-stimulated generation of inflammatory mediators compared to the common RAGE 82Gly allele[116]–[120]. It affects the structure of the receptor protein, influencing its cleavage by some proteases resulting in decrease proteolysis of RAGE[121].

Our study indicated that the genetic polymorphisms in the RAGE gene (rs2070600G>A) neither associated with serum RAGE and CRP nor the risk for Aspirin resistance.

As we mentioned there is no study till now relates the RAGE gene polymorphism with the risk of Aspirin resistance to compare its the results with ours.

However, regarding to the correlation with CRP, several studies from different settings showed presence of significant positive correlation between Gly82Ser and CRP[116]–[120], [122].

We presume that lack of relationship between this polymorphism and Aspirin resistance in our patients may due to several reasons; First: the multifactorial nature of Aspirin resistance phenomena; Second: this polymorphism may have little or no detectable contribution to Aspirin resistance; Third: it was single-locus-based, so haplotype-based effects may be another issue.

Finally: excluding the possibility of gene-gene and gene-environment interactions, in other word it possible that the potential role of RAGE gene is diluted or masked by other gene-gene or gene-environment interactions.

Although our study did not specify the role of Gly82Ser as a risk factor, for more accurate results, an increase in sample size, and dealing with the above presumptions are recommended.

CONCLUSION

The prevalence of aspirin resistance in studied Iraqi patients was relatively high and risky so the specialist should be aware about such issue and its fatal consequences.

when the roles of RAGE in the complex and multifactorial inflammatory balance will be deeply defined, it could be used as markers of disease severity and/or response to treatment.

Our study didn't show significant association between Gly82Ser variant with the risk of Aspirin resistance, in the same time there was strong association between serum level of RAGE and Aspirin resistance, and strong positive correlation between RAGE expression and CRP serum level, suggesting RAGE expression may contribute to the systemic inflammatory and oxidative stress state accompanying Aspirin resistance.

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