Research

Recurrent Coronary Artery Disease Due to Acetylsalicylic Acid Resistance May Be Related to COX-1 and COX-2 Mutations

Asetilsalisilik Asit Direnci Nedeni ile Oluşan Rekürren Koroner Arter Hastalığı, COX-1 ve COX-2 Mutasyonları ile İlişkili Olabilir

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ABSTRACT

Objective: Acetylsalicylic acid (ASA) is a commonly used antiplatelet drug for the treatment of coronary artery disease (CAD). However, in some patients recurrent CAD occurs due to ASA resistance (AR). This condition may be related to some genetic factors. Therefore, this study aims to investigate the effects of cyclooxygenase (COX)-1 and COX-2 mutations on recurrent CAD due to AR.

Methods: Hundred CAD patients taking 100 mg ASA daily for 2 years were enrolled to the study. The patients were divided into two groups according to their recurrent CAD status. Forty-eight patients with recurrent CAD due to AR and 52 patients without recurrent CAD were selected to ASA resistant (AR+) and ASA non-resistant (AR-) group, respectively. AR was confirmed by platelet aggregation testing. Risk factors related to recurrent CAD were also obtained. After DNA was isolated from peripheral blood, rs1330344 variation in *COX-1* and rs20417 variation in *COX-2* were determined using real-time polymerase chain reaction. Results were evaluated statistically.

Results: COX-1 and COX-2 mutations were mostly detected in the AR+ group however these data were not found statistically significant. Nevertheless, C allele of COX-2 was found statistically high in the AR+ group (67.9%) (p=0.023). Additionally statistically significant associations were found between high total cholesterol and low density lipoprotein cholesterol levels with the GC genotype of COX-2.

Conclusion: It was suggested a relation between COX-2 mutations and recurrent CAD due to AR. Similar studies with a large population must explain the mechanisms governing the association of COX-1 and COX-2 genotypes and response to ASA in recurrent CAD patients.

Keywords: ASA resistance, recurrent CAD, COX-1, COX-2

ÖZ

Amaç: Asetilsalisilik asit (ASA), koroner arter hastalığının (KAH) tedavisinde yaygın olarak kullanılan antitrombotik ilaçlardan biridir. Ancak bazı hastalarda ASA direncine (AR) bağlı olarak rekürren KAH oluşur. Bu durum bazı genetik faktörlerle ilişkili olabilir. Bu nedenle bu çalışmanın amacı, siklooksijenaz (COX)-1 ve COX-2 mutasyonlarının AR'ye bağlı rekürren KAH oluşumu üzerine etkilerini araştırmaktır.

Gereç ve Yöntem: İki yıl boyunca günlük 100 mg ASA kullanan 100 KAH hastası çalışmaya alındı. Hastalar tekrarlayan KAH durumlarına göre iki gruba ayrıldı. AR'ye bağlı rekürren KAH gözlenen 48 hasta ASA dirençli (AR+) grubuna, rekürren KAH gelişmeyen 52 hasta ise ASA dirençli olmayan (AR-) grubuna dahil edildi. AR, trombosit agregasyon testi ile ölçüldü. Ayrıca diğer risk faktörleri ile ilgili bilgiler temin edildi. Periferik kandan DNA izole edildikten sonra *COX-1* genindeki rs1330344 ve *COX-2* genindeki rs20417 mutasyonlarının varlığı gerçek zamanlı polimeraz zincir reaksiyonu yöntemi kullanılarak incelendi. Sonuçlar istatistiksel olarak değerlendirildi.

Bulgular: COX-1 ve COX-2 mutasyonları en çok AR+ grubunda tespit edilmiş olmasına rağmen, bu sonuç istatistiksel olarak anlamlı bulunmadı. Bununla birlikte, AR+ grubunda COX-2'nin C alleli istatistiksel olarak anlamlı düzeyde yüksek bulundu (%67,9) (p=0,023). Ek olarak yüksek total kolesterol ve düşük yoğunluklu lipoprotein kolesterol düzeyleri ile COX-2'nin GC genotipi arasında istatistiksel olarak anlamlı ilişkili olduğu belirlendi.

Sonuç: COX-2 mutasyonları ile AR'ye bağlı rekürren KAH arasında bir ilişki olduğu düşünülmektedir. Rekürren KAH gözlenen hastalarda COX-1 ve COX-2 genotiplerinin ilişkisini ve ASA'ya yanıtı etkileyen mekanizmaları açıklamak için daha fazla hasta ile benzer çalışmaların yapılmasına ihtiyaç vardır.
Anahtar Kelimeler: ASA direnci, rekürren KAH, COX-1, COX-2

INTRODUCTION

Coronary artery disease (CAD) is a significant health problem and its complications lead to mortality and disability in the world (1). CAD causes more than 7 million people deaths every year worldwide (2). CAD is a complex disease related to genetic and environmental factors (3).

Acetylsalicylic acid (ASA) is an antiplatelet agent and commonly used for treating cardiovascular disease (4). ASA can decrease mortality and adverse cardiovascular events in patients with CAD (5,6). However, it is thought that approximately half of the patients do not benefit from ASA effectively (7). This condition is known as ASA resistance (AR). AR involves the development of thrombotic vascular events despite aspirin treatment. The heritability of this disorder is thought to be approximately 50% (3). Therefore, investigation of the genetic basis of this disorder due to AR is important for finding new treatment approaches.

Patients who have AR are unable to respond to treatment for CAD as a result of recurrent cardiovascular events are observed in these patients (8-11). ASA irreversibly inhibits platelets by acetylating COX-1 and COX-2 enzymes in platelets (12,13). *COX* genes are related to ischemic stroke (14,15).

The *COX-1* gene contains 10 introns and 11 exons. It is located on chromosomes 9q32-q33.3. COX-1 regulates blood coagulation and platelet function in the body. COX-2 contains 9 introns and 10 exons. It is located on chromosomes 1q25.2-q25.3. COX-2 is found generally in the nuclear membrane and exists in platelets and vascular endothelial cells (16).

Although *COX-1* and *COX-2* genes are related to AR, studies on this subject are insufficient. Therefore in this study the relation between COX-1 and COX-2 variations with AR in recurrent CAD patients were investigated.

The etiology of AR also includes some other factors such as smoking, poor diet, diabetes mellitus, gender, non-compliance with the amount of drug use, hypertension and excessive COX-2 production in platelets (17-19). There may also be some other factors that lead to unsuccessful ASA therapy (17). Therefore in this study the relation between COX-1, COX-2 variations, and other risk factors with recurrent CAD due to AR was also investigated.

METHODS

Patient Characteristics

A hundred patients with CAD who have applied University of Health Sciences Turkey, Umraniye Training and Research Hospital, Cardiology Polyclinic and taking 100 mg ASA daily for 2 years (2018-2020) were enrolled to the study. The patients were divided into two groups according to their recurrent CAD status. Forty-eight patients with recurrent CAD were selected to the AR+ group and 52 patients without recurrent CAD were selected to the AR- group. Patients diagnosed with recurrent CAD were selected from patients who had previously undergone percutaneous coronary intervention (PCI). The recurrent CAD group was determined as those who needed revascularization again with PCI after the first procedure. Patients who used ASA with other antiaggregant or anticoagulants, were allergic to ASA and stopped ASA for any reason for 2 years were excluded from the study. The information regarding the risk factors such as smoking, alcohol consumption was also obtained. This study is an experimental study. This study protocol was approved by the Institutional Ethics Committee of University of Health Sciences Turkey, Umraniye Training and Research Hospital, Istanbul, Turkey (decision no: 139, date: 24.07.2019). Each individual was informed about the study and written informed consent was obtained from each participant. The study was conducted in accordance with the relevant regulations of the Ministry of Health.

Platelet Aggregation Testing

AR was confirmed by platelet aggregation testing. Platelet aggregation studies were conducted in whole blood lumiaggregometer (Chronolog Corporation, Model 560-Ca). AR was defined as a mean aggregation of ≥20% with 0.5 mg/mL arachidonic acid and a mean aggregation of ≥64% with 5 µM adenosine diphosphate (20).

Blood Sampling and Genotyping

DNA samples of 100 patients were isolated from peripheral blood sample using QIAamp DNA Blood Mini kit (Qiagen, GmBH, Hilden, Germany). DNA concentrations were measured using a Nanodrop spectrophotometer (Thermo Scientific, Foster City, CA, USA). Real-time polymerase chain reactions (PCR) for COX-1 (rs1330344) (-1676 T>C) and COX-2 (rs20417) (-765G>C) were performed using 7500 Fast Real-Time PCR System (Applied Biosystems, Foster

City, CA, USA). The reaction was performed according to the manufacturer's instructions.

Statistical Analysis

Statistical Package for the Social Science 23.0 was performed for statistical analysis. Normal distribution assumption was checked with the Kolmogorov-Smirnov test. Two independent samples t-tests was used to compare continuous variables' means between two groups which were normally distributed. Kruskal-Wallis tests were performed to investigate the difference between genotypes and risk factors (which are not normally distributed). If there were statistically significant differences for pairwise comparison, Mann-Whitney U test was performed and Bonferroni correction was applied to p values. P values less than 0.05 (p<0.05) were considered statistically significant.

RESULTS

Study Population

Table 1 shows the baseline characteristics of the study population. When the characteristics were compared between groups; gender, fasting blood glucose level, high density lipoprotein (HDL) and hypertension were found statistically significant in AR+ group (p<0.05).

COX-1 and COX-2 Genotyping

Table 2 shows the genotype distribution of groups. When groups were compared with each other, however heterozygote and homozygote variations were mostly found in the AR+ group for both COX-1 and COX-2, the relation between groups were not found statistically significant.

Allele Frequencies of COX-1 and COX-2 Variations

Table 3 shows allele frequencies of *COX-1* and *COX-2* genes in study groups. When groups were compared with each other, the presence of C allele of COX-2 was found statistically high in the AR+ group (p=0.023).

Relation Between Risk Factors and Polymorphisms

When the relation between risk factors and variations were investigated, it was found that the heterozygote genotype of COX-2 is associated with high total cholesterol and Low density lipoprotein (LDL) levels (p<0.05). Table 4 shows the relation between risk factors and mutations.

DISCUSSION

CAD is one of the most known heart disease and leads to death in the world. Considering factors such as unhealthy life, CAD rates are also increasing in low and middle income countries (21). Due to recurrent CAD, adverse outcomes

Table 1. Baseline characteristics of the study population

Baseline characteristics		Groups (number of participants)		p-value
		AR- group (n=52)	AR+ group (n=48)	
Age (years)		59.44±10.52	59.60±10.28	0.938
Height (cm)		169.12±28.45	166.71±7.37	0.414
Weight (kg)		78.09±10.23	80.31±10.94	0.231
Body mass index		28.66±3.79	28.89±3.79	0.6
Gender	Female	29 (55.8%)	15 (31.3%)	0.014*
	Male	23 (44.2%)	33 (68.8%)	
Fasting blood glucose level (mg/dL)		106.62±36.43	117.85±39.01	0.002*
Total cholesterol (mg/dL)		191.04±42.43	203.27±62.25	0.588
LDL (mg/dL)		111.69±41.09	130.39±54.69	0.137
HDL (mg/dL)		51.31±10.91	46.19±8.82	0.012*
Triglyceride (mg/dL)		144.39±92.75	154.67±90.59	0.28
Diabetes mellitus (%)		9 (17.3%)	11 (22.9%)	0.484
Hypertension (%)		24 (46.2%)	34 (70.8%)	0.012*
Smoking (%)		26 (50%)	31 (64.6%)	0.141
Alcohol consumption (%)		3 (5.8%)	5 (10.4%)	0.475

LDL: Low density lipoprotein, HDL: High density lipoprotein, COX: Cyclooxygenase, AR-: ASA non-resistant, AR+: ASA resistant, ASA: Acetylsalicylic acid, *p<0.05

such as death, heart failure, stroke, malignant arrhythmia can be seen more often (22,23). Therefore, it is critical to identify preventable causes of recurrent CAD and to take action against them. The frequency of recurrent cardiovascular events due to AR also varies between countries (24).

A meta-analysis showed a 25% reduction in serious vascular events in high-risk patients with CAD using ASA (10). ASA prevents the conversion of arachidonic acid to thromboxane TXA2 by irreversibly inhibiting COX-1. This inhibition occurs by acetylation of the serin-530 residue located in the active site of COX-1 (25,26). Irreversible enzyme inhibition causes complete COX-1 inhibition with a daily dose of ASA (26). ASA can also reduce the risk of ischemic events by 22% in patients with atherothrombosis (7). However, recurrent cardiovascular events have still seen at a high rate in patients who use ASA (27). Therefore, identification of genetic or environmental factors that may cause recurrent CAD is important for the new therapeutic approaches. Therefore in this study the relation between COX-1, COX-2 variations, and other risk factors related to AR in recurrent CAD patients were investigated.

Table 2. COX-1 and COX-2 genotyping results of groups

Gene names accession number of variations and genotype distributions	Groups (number of participants)		p-value
	AR- group (n=52)	AR+ group (n=48)	
COX-1 (rs1330344) (-1676 T>C)			
TT	42 (80.8%)	32 (66.7%)	
TC	10 (19.2%)	15 (31.3%)	0.202
CC	0 (0%)	1 (2.1%)	
COX-2 (rs20417) (-765G>C)			
GG	44 (84.6%)	32 (66.7%)	
GC	7 (13.5%)	13 (27.1%)	0.103
CC	1 (1.9%)	3 (6.3%)	

*p<0.05, COX: Cyclooxygenase, AR-: ASA non-resistant, AR+: ASA resistant, ASA: Acetylsalicylic acid

Table 3. Allele frequencies of COX-1 and COX-2 in study groups

Gene names accession number of variations and alleles	Groups and allele distributions		p-value
	AR- group (n=52)	AR+ group (n=48)	
COX-1 (rs1330344) (-1676 T>C)			
T	94 (90.4%)	79 (82.3%)	0.094
C	10 (9.6%)	17 (17.7%)	
COX-2 (rs20417) (-765G>C)			
G	95 (91.3%)	77 (80.2%)	**0.023***
C	9 (8.7%)	19* (19.8%)	

*p<0.05, COX: Cyclooxygenase, AR-: ASA non-resistant, AR+: ASA resistant, ASA: Acetylsalicylic acid

Table 4. Relation between risk factors and mutations

Risk factors-variations relationship	Genotype distributions			p-value
	Wild type	Homozygous mutation	Heterozygous mutation	
COX-2 (rs20417)				
Total cholesterol	188.89±50.39*	188.25±43.57	229.1±54.11*	0.01*
LDL	112.13±44.57*	114.5±41.46	154.35±52.75*	0.004*

*p<0.05. LDL: Low density lipoprotein, COX: Cyclooxygenase

COX is an enzyme that is responsible for the synthesis of prostaglandins (PGs) and platelet generation of TXA2. PGs produced by COX-2 can also be synthesized by COX-1 (8). rs1330344 (-1676 T>C) of COX-1 was found to contribute significantly to the occurrence of ischemic stroke. On the one hand, it has been noted that the TT genotype of rs1330344 can reduce ischemic stroke susceptibility and cardioembolic stroke or small vessel occlusion (16). In one study, it was found that the frequency rates of alleles in the COX-1 C50T were 8.6%, therefore this variation may influence the effect of ASA (8). A842G, C22T, G128A, C644A, and C714A are mostly detected variations in the COX-1 and they are related to ASA response (28). In another study, low-dose ASA irreversibly acetylates COX-1 and reduce platelet activity by inhibiting the production of thromboxane A2 (29). In our study, however COX-1 rs1330344 variation was mostly found in the AR+ group, the relation between groups was not found statistically significant (Table 2).

COX-2, induced by cytokines in response to inflammatory stimuli, has been expressed on endothelial cells and macrophages (8). rs20417 SNP is located in the promoter region of COX-2 (-765G>C). This locus mutation in the *COX-2* gene can change the promoter activity and affect the expression of COX-2 (16). In a study, a significant relationship was found between rs20417 polymorphism and CAD (30). In another study it was found that the frequency rate of C allele in the COX-2 rs20417 is 21.3% (8). Therefore, it was suggested that the variation of the *COX-2* gene influences the effect of ASA. Some metabolic factors (reduced absorption or increased metabolism of ASA) may also cause AR. Some studies have suggested that the COX-2 variant increases the risk of AR (8,31). The *COX-2* gene is induced by the activation of the signal transduction pathway and COX-2 protein is covalently acetylated by ASA. ASA activity can be measured using serum TXB2 or urine 11-DH-TXB2 (TXA2 pathway endproducts). Unlike other results, the COX-2 -765G>C variant after ASA treatment causes a high decrease in serum and urine 11-dehydrothromboxane B_2 (11-DH-TXB2) levels (28). In our study, COX-2 (33.3%) variation was mostly detected in the AR+ group, however the relation between groups was not found statistically significant (Table 2). When allel frequencies were compared between groups, C allele of COX-2 was found statistically high in AR+ group (p<0.05) (Table 3). Therefore, it was suggested a relation between COX-2 mutations and recurrent CAD due to AR.

One study showed a higher prevalence of AR in patients with acute coronary syndrome than healthy individuals. AR was more prevalent in patients with smokers and low HDL cholesterol. However, no significant difference was found for gender, age and hypertension (11). In other studies any relation was not detected for total cholesterol and LDL cholesterol level with COX-2 variations however in our study, we also found that high total cholesterol and LDL cholesterol levels are related to the GC genotype of COX-2 (Table 4).

CONCLUSION

Similar studies with a large population must explain the mechanisms governing the association of *COX-1* and *COX-2* genotypes and response to ASA in recurrent CAD patients. Detection of AR may be useful in preventing recurrent CAD and reducing mortality and morbidity associated with recurrent CAD.

ETHICS

Authorship Contributions

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