

# Aspirin resistance and ischemic heart disease on Iranian experience

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## Abstract

**Background:** Coronary artery disease (CAD) and myocardial infarction are the most common causes of mortality and morbidity all over the world. Aspirin resistance is an important part of therapeutic failure in patients who experience several atherosclerotic events despite aspirin therapy. Different studies have reported aspirin resistance between 5% and 45% all over the world.

According to different responses to aspirin therapy in countries and lack of adequate studies on aspirin resistance in Iran, this study was designed for evaluation of aspirin resistance in ischemic patients.

**Materials and Methods:** Total 170 patients with documented coronary artery stenosis with were enrolled in this cross-sectional prospective study. Two cc urine samples were obtained from all the subjects. Then a questionnaire including questions about major risk factors (hypertension, diabetes, hyperlipidemia, obesity and smoking) was completed for each patient. Thromboxane B2 level in urine was measured two times for each patient by one kit of via ELISA method. Gensini modified was used for assessment of severity of coronary arteries involvement. Data were analyzed via SPSS 16. with general linear model (univariate).

**Results:** 75.3% of studied patients were aspirin resistant. There was significant relationship between angiography score and aspirin resistance ( $P < 0.001$ ). Our results also showed that aspirin resistance is more common in studied women than men ( $P = 0.003$ ). Significant correlation was observed between diabetes and aspirin resistance in studied subjects ( $P = 0.023$ ).

**Conclusion:** Our study showed aspirin resistance in a sample of Iranian ischemic patients is so prevalent which is higher than other studies in another communities and also aspirin resistance is more common in patients with severe CAD.

**Key words:** Aspirin resistance, coronary artery disease, thromboxane B2 level

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## INTRODUCTION

Coronary artery disease (CAD) is the most common causes of mortality and morbidity all over the world. Aspirin is a very important and inexpensive medication for secondary prevention from atherosclerotic events. Many studies have shown that aspirin can prevent from 25% of atherosclerotic events.<sup>[1,2]</sup>

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Arachidonic acid is converted into thromboxane A2 via cyclo-oxygenase (COX) enzyme within the platelets. Thromboxane A2 prevents from platelet aggravation via fibrin receptor inhibition on the platelet surface. Aspirin acetylate catalytic region of COX enzyme and prevents from conversion of arachidonic acid to thromboxane A2.<sup>[3,4]</sup>

Biochemical aspirin resistance is defined as stable platelet activity which is calculated via activity measurement tests and occurs despite correct use of aspirin in common therapeutic dose.<sup>[5]</sup> Aspirin resistance is an important part of therapeutic failure in patients who experience several atherosclerotic events despite its use.<sup>[6,7]</sup> Studies showed different factors which are effective on aspirin resistance like correct use of drug, dosage, drug interactions and genetic and racial polymorphism.<sup>[8-11]</sup>

A growing body of evidences show that up to 70% of aspirin-takers are at risk of atherothrombotic complications because of aspirin resistance.<sup>[12]</sup> Kim *et al.* study showed 14% aspirin resistance in patients with CAD and ischemic cerebrovascular disease.<sup>[13]</sup> Shen *et al.* study showed 21% aspirin resistance in asymptomatic subjects (30% in women and 16% in men).<sup>[14]</sup> Pinto Slottow *et al.* reported 23% aspirin resistance in patients with stent thrombosis (ST).<sup>[15]</sup> Sanioglu *et al.* showed 60% resistance in postoperative patients who underwent elective coronary artery bypass graft (CABG).<sup>[16]</sup> Acikel *et al.* reported 29.9% laboratory-defined aspirin resistance in patients with unstable angina.<sup>[17]</sup> Akhtar *et al.* study showed 12% aspirin resistance in patients with stable CAD.<sup>[18]</sup>

A probable reason for different responses to aspirin in different races may be due to polymorphism in cyclooxygenase 1 and 2 genes and other metabolites of arachidonic acid.<sup>[11]</sup>

Diagnosis of aspirin resistance is important in different communities because its impact on therapeutic costs and therapeutic response. Thromboxane B2 is a metabolite of thromboxane A2 in urine which shows the *in vitro* response to aspirin and is a simple, non-invasive, and inexpensive.<sup>[19,20]</sup>

According to different responses to aspirin therapy in different countries and lack of adequate studies on aspirin resistance in Iran, this study was designed for evaluation of aspirin resistance in Iranian ischemic patients. We also assessed the correlation between its resistance with severity of coronary artery involvement which hasn't report in previous studies.

## MATERIALS AND METHODS

This study was a cross-sectional prospective study on patients with documented coronary artery stenosis via angiography which were admitted in University hospitals in Isfahan.

Inclusion criteria were: Age between 40 and 70 years, 75-100 mg daily aspirin use, significant stenosis in at least one coronary artery (more than 75% of intravessel diameter). Exclusion criteria were: Hb <8 g/dl, platelets <150000/dl, non-steroid anti-inflammatory medications, plavix and ticlopidin use, surgery during last 2 weeks, malignancy or acute inflammatory disease, heparin and warfarin use, emergency angiography. One hundred and seventy participants were selected via a simple sampling method among hospitalized patients with inclusion criteria. Written consents were obtained from all of the participants.

Subjects were divided to three groups (stable angina, unstable angina, and myocardial infarction (MI)) by history and medical record. Significant severity of coronary artery stenosis has been confirmed by three cardiologists after angiography and showed as quantitative numbers with the Gensini method.<sup>[21]</sup>

Two milliliters urine samples were obtained from all the subjects. Then a questionnaire was filled about risk factors (hypertension, diabetes, hyperlipidemia, and smoking) for each patient. Hypertension was defined according to WHO definition as systolic blood pressure  $\geq 140$  mmHg and diastolic blood pressure  $\geq 90$  mmHg or anti-hypertensive medications use.<sup>[22]</sup> Diabetes was defined as fasting blood sugar  $\geq 126$  mg/dl or 2 h post prandial blood sugar  $>200$  or use of hypoglycemic agents.<sup>[23]</sup> Hyperlipidemia was defined as total cholesterol  $>200$  mg/dl or use of anti-cholesterol agents or triglyceride  $>200$  mg/dl or use of anti-triglyceride agent.<sup>[24]</sup> Cigarette smokers were defined as individuals who smoked more than 10 packets in last year and were between 20 and 60 year old and non-smokers were defined as individuals who never smoked any cigarettes.<sup>[25]</sup>

Thromboxane B2 level in urine was measured via the ELISA method. A diluted sample of subjects urine, a control sample and a solution of purified thromboxan attached to alkaline phosphatase and monoclonal antibody of mouse were put in one container and a chromogen agent was added to the container for assay.<sup>[26]</sup> For more accuracy of the results, ELISA was done two times, with two separated same kits for each participant. Intensity of produced color in the container had reverse correlation with concentration of thromboxan in the urine sample.<sup>[26]</sup>

The modified Gensini method was used for assessment of severity of coronary arteries involvement (coronary angiography score).<sup>[27]</sup> Aspirin resistance was described by quantitative numbers and based on the definition in the laboratory kit, numbers  $\geq 1700$  ng/dl were considered as aspirin resistance. Data were analyzed via SPSS 16; general linear model (univariate) was used for assessment of correlation between aspirin resistance with CAD risk factors and coronary artery involvement. A *P* value less than 0.05 was considered as significant.

## RESULTS

Total 170 subjects were enrolled in this study. Table 1 shows characteristics of study participants. Most of the studied subjects were male (53.5%) and more than half of them (55.9%) had chronic stable angina.

Results of this study showed that 128 of 170 participants (75.3%) had aspirin resistance.

Correlation between aspirin resistance with CAD risk factors was assessed too [Table 2]. Our results showed, aspirin resistance is significantly higher in studied women ( $P=0.003$ ,  $B=768.697$ ). Also, aspirin resistance was significantly higher in diabetic subjects ( $P=0.023$ ,  $B=604.353$ ). There wasn't any significant relationship between aspirin resistance and other CAD risk factors in this study.

Results of our study showed that patients with more

sever CAD (according to Gensini score), had more resistance to aspirin ( $P<0.001$ ,  $CI=\pm 3.712$ ,  $B=7.485$ ,  $CI=\pm 3.712$ ) [Figure 1].

## DISCUSSION

In this study, we estimated aspirin resistance in patients with coronary artery stenosis about 75.3%. This percentage is higher than the results of previous studies.

Several studies have reported aspirin resistance in different communities between 5.5% and 60% according to definition and measured parameters.<sup>[28]</sup>

Christiaens *et al.* study showed 29% aspirin resistance in patients with stable angina under treatment with aspirin.<sup>[29]</sup> Akay *et al.* reported 27% aspirin resistance in healthy subjects.<sup>[30]</sup>

Recently, a growing body of evidences show that about 70% of aspirin-takers are at risk of atherothrombotic complications.<sup>[12]</sup>

Shen *et al.* study showed 21% aspirin resistance in asymptomatic subjects (30% in women and 16% in men).<sup>[14]</sup>

Sanioglu *et al.* showed 60% aspirin resistance in

**Table 1: Characteristics of study participant**

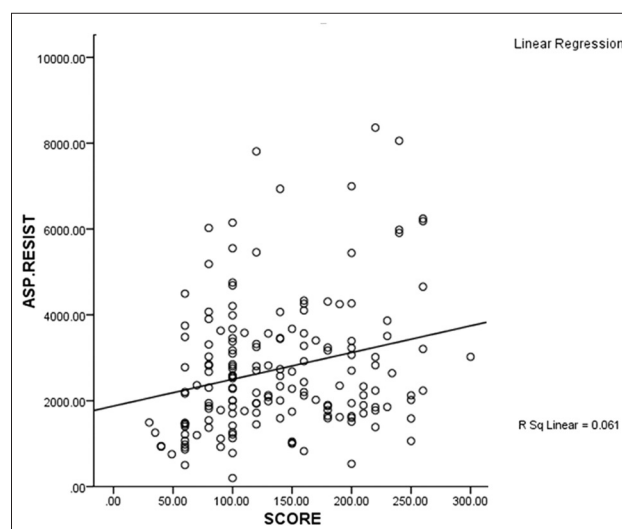
Patient characteristics	Participants number (percent) or Mean $\pm$ SD
Age (year)	60.42 $\pm$ 8.43
Female sex	79(46.5)
Hypertension	100(58.8)
Diabetes	43(25.3)
Hyperlipidemia	80(47.1)
Patients Category	
Myocardial infarction	44(25.9)
Unstable angina	50(29.4)
Chronic stable angina	95(55.9)
Aspirin resistance	2736.69 $\pm$ 1540.50
Angiography score	137 $\pm$ 60.00

Values are mean  $\pm$  standard deviation or n(%).MI, indicates myocardial infarction;USA,unstable angina;CSA,chronic stable angina;CABG,coronary artery bypass graft;PCI,percutaneous coronary intervention;HTN, hypertension,DM,diabetes mellitus; Angiography Score.severity of coronary artery stenosis.

**Table 2: Correlation between aspirin resistance and mean of baseline characteristics in study participants**

Aspirin resistance*	Sex (male)	Age	Chronic stable angina	Unstable angina	Hypertension	Diabetes	Hyperlipidemia	Myocardial infarction	Smoking
B reference**	-768.69	0.74	396.47	265.90	-270.05	604.35	101.02	-768.05	476.42
CI***	$\pm 499$		$\pm 637$	$\pm 671$	$\pm 471$	$\pm 518$	$\pm 459$	$\pm 551$	$\pm 621$
P value****	0.003*	0.958	0.220	0.435	0.267	0.023*	0.665	<0.001	0.132

\*Dependent variable: aspirin resistance \*\*B reference: no disease or risk factor \*\*\*CI: confidence interval \*\*\*\*P value <0.05 is considered significant.



**Figure 1:** Correlation between angiography score and aspirin resistance.

postoperative patients who underwent elective CABG.<sup>[16]</sup>

Singla *et al.* assessed laboratory aspirin resistance in patients with type II diabetes and reported 7.2% aspirin resistance and 39.1% semi-aspirin resistance.<sup>[31]</sup>

Zimmermann *et al.* study reported Laboratory aspirin resistance up to 60% in patients after stroke or peripheral arterial disease, up to 70% in stable coronary heart disease, and even up to 80% in acute MI.<sup>[32]</sup>

Aspirin-resistance percent (75.3%) in our study is close to results of Zimmermann *et al.* study (70% in stable CAD and 80% in MI)<sup>[32]</sup>, may be because of similar sample group disease the subjects have of both studies.

These differences between our results and other studies may have several reasons like: Racial differences and genetic polymorphism between different communities; we didn't separate aspirin-resistant and semi-aspirin resistant patients from each other like some studies.

Another probable reasons for different percent of aspirin resistance in our study is more non-smokers (80%) in our participants. Studies have reported that biochemical aspirin resistance is more common in non-smokers.<sup>[33]</sup>

The prevalence of aspirin resistance depends on the method of testing<sup>[34]</sup> and this may be another reason for different reported prevalence of aspirin resistance between different studies.

Another study in Iran showed that approximately 49.2% patients were resistant to ASA, 15.3% borderline response, and 35.5% were sensitive to ASA. Acetyl salicylic acid resistance same as our study was present in a high number of patients with chronic stable angina. Moreover, advanced age and smoking had a direct influence on the aspirin resistance.<sup>[35]</sup>

The top point of this study was that aspirin resistance was measured with the ELSIA method which is the most accurate method for measurement of aspirin resistance and to get more accurate results, in each patient aspirin resistance was checked twice with two separate same kits.

Also in this study, we assessed the correlation between aspirin resistance with severity of CAD which hasn't been assessed in the previous studies.

## CONCLUSION

Our results showed that in patients with coronary

artery stenosis, aspirin resistance has significant correlation with diabetes, female sex, and severity of coronary artery stenosis.

According to high prevalence of aspirin resistance in our community based on the current study and considering potential cardiovascular complications and their mortality and morbidity due to aspirin resistance, it is important to assess the aspirin sensitivity as a screening method for evaluation of patients participated to aspirin therapy. In this way, we can increase anti-platelet effect with an alternative or adjuvant medication and benefit from the effects of this medication in treatment of patients with CAD.

## Study limitations

Our study was some limitations. Aspirin use in this study was based on response to a questionnaire and wasn't confirmed by pill count or salicylate level, we didn't separate aspirin-resistant and semi-aspirin resistant patients from each other.

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