Relationship Between Endothelial Functions and Acetylsalicylic Acid Resistance in Newly Diagnosed Hypertensive Patients

Tayfun Sahin, MD; Umut Celikyurt, MD; Bilal Geyik, MD; Gökhan Oner, MD; Teoman Kilic, MD; Ulas Bildirici, MD; Guliz Kozdag, MD; Dilek Ural, MD

Department of Cardiology (Sahin, Celikyurt, Oner, Kilic, Bildirici, Kozdag, Ural), Medical Faculty of Kocaeli University, Kocaeli, Turkey; Department of Cardiology (Geyik), Medical Faculty of Trakya University, Edirne, Turkey

ABSTRACT

Background: We aimed to investigate the effects and dose dependency of aspirin on endothelial functions and prevalence of aspirin resistance in newly diagnosed hypertensive patients without previous drug therapy and development of cardiac complications.

Hypothesis: Acetylsalicylic acid improves endothelial function.

Methods: Fifty-eight hypertensive patients and 61 healthy subjects in the control group were included in the study. Endothelial functions of the patient and control groups were evaluated with brachial artery examination. Patient and control groups were divided into 2 groups. A total of 100 mg and 300 mg of aspirin were given to the separate groups for 1 week. After 1 week, endothelial functions were reevaluated and aspirin resistance examined with a platelet function analyzer (PFA-100; Dade Behring, Marbourg, Germany).

Results: Baseline flow-mediated dilatation (FMD) change percent in hypertensive patients was 9.8%, and it was significantly lower than in the control group (12%) ($P < 0.001$). Frequency of acetylsalicylic acid (ASA) resistance was 20% and 26% in control and hypertensive patient groups, respectively ($P =$ not significant). ASA resistance was 28% and 24% in 100 mg and 300 mg in hypertensive patients, respectively ($P =$ not significant). FMD change percent increased both in the control and hypertensive groups after ASA treatment from 12.4% to 13.3% and 9.8% to 11.9%, respectively. FMD percentage change was significantly increased in hypertensive patients irrespective of ASA resistance ($P = 0.02$, for ASA resistance [+]; $P < 0.012$, for ASA resistance [−]).

Conclusions: Endothelial functions were impaired more in hypertensive patients compared to the control group. Endothelial functions were improved with all ASA doses in hypertensive patients irrespective of ASA resistance.

Introduction

The vascular endothelium regulates cardiovascular homeostasis and blood pressure (BP) through the release of nitric oxide (NO).^{1,2} NO is an anti-inflammatory, antithrombotic, and antiproliferative agent that limits expression of adhesion molecules and chemotactic factors on endothelium, and inhibits platelet aggregation and proliferation of smooth muscle cells.^{3–5}

Endothelial dysfunction and decreased NO bioavailability have been reported as the first stages in the development of atherosclerosis.^{5–8} In hypertensive patients, basal NO activity is decreased and acetylcholine-induced endothelium-dependent vasodilation is impaired in the forearm circulation.^{9–11} Several studies revealed that endothelial dysfunction is associated with a high incidence

of cardiovascular events.^{6,7,12,13} A significant relationship between endothelial dysfunction and diastolic dysfunction, and hypertension has been demonstrated previously.¹⁴

Acetylsalicylic acid (ASA) (aspirin) is a nonsteroidal anti-inflammatory and antiaggregative drug. ASA inhibits cyclooxygenase, which is responsible for arachidonic acid metabolism and prostaglandin production. It is used to prevent arterial thrombosis, cerebral strokes,¹⁵ or infarction.^{16,17}

Hypertension is a risk factor for various cardiovascular diseases.¹⁸ Systemic inflammation and endothelial activation play role in the development of hypertension.¹⁹ The activation of the sympathetic nervous system, renin-angiotensin-aldosterone system, and inflammatory mediators are involved in the pathophysiology of hypertension. Systemic inflammation impairs endothelium-dependent dilatation. Therefore, pretreatment with an anti-inflammatory dose of aspirin may protect from this endothelial dysfunction.²⁰

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Some studies revealed that thromboembolic events occurred in some patients who were under therapeutic doses of aspirin. The absence of the expected antiaggregant effect of aspirin is defined as aspirin resistance, aspirin insensitivity, or unresponsiveness to aspirin. The incidence of aspirin resistance was reported as 8% to 45% in the literature.²¹

In this study, we investigated the effects and dose dependency of aspirin on endothelial functions and prevalence of aspirin resistance in newly diagnosed hypertensive patients without previous drug therapy and development of cardiac complications and healthy controls.

Methods

Study Design, Patients, and Protocol

Fifty-eight hypertensive patients admitted to our university clinic with the diagnosis of new onset and untreated essential hypertension with respiratory reversal in early diastolic peak flow velocity (E)/late diastolic peak flow velocity (A) flow were included in the study. Sixty-one normotensive subjects with normal cardiovascular histories, physical examinations, and normal diastolic function without respiratory changes in mitral flow served as the control group. To be included in this study, patients were required to have newly diagnosed and previously untreated mild to moderate hypertension according to European Society of Hypertension–European Society of Cardiology criteria (BP > 140/90 mm Hg).¹⁸ Patients with severe hypertension (BP ≥ 180/100 mm Hg) or who had received prior antihypertensive therapy were excluded. Patients with bradycardia (heart rate < 60 beats/minutes), sick sinus syndrome, atrioventricular block, atrial fibrillation or other arrhythmias, valvular heart disease, coronary artery disease, left ventricular ejection fraction < 50%, inappropriate echocardiographic window, or left ventricular hypertrophy were also excluded. Additional exclusion criteria included a diagnosis of diabetes mellitus (glycosylated hemoglobin > 6.5%), renal impairment (serum creatinine > 2.2 mg/dL), hepatic insufficiency, and peripheral arterial disease. Hypersensitivity to aspirin, the use of other nonsteroidal anti-inflammatory drugs, oral contraceptive agents, platelet count lower than 140,000/μL or higher than 450,000/μL, hemoglobin level lower than 10 g/dL, the presence of a myeloproliferative disease, and history of individual or familial bleeding diathesis were also excluded from the study. All study participants were informed about the study and gave written consent before study procedures. The study was approved by the local ethical committee. This study was conducted in accordance with latest version of Declaration of Helsinki.

All patients underwent a clinical, physical, and electrocardiographic examination. Resting BP was measured with a mercury sphygmomanometer after 10 minutes of rest, and repeated twice at 3-minute intervals. Korotkoff phase I and V sounds were used for systolic BP and diastolic BP determination, with the average of the 3 measurements taken.

Endothelial functions of the patient and control groups were evaluated with brachial artery examination. Patient and control groups were divided into 2 groups. A total of 100 mg and 300 mg aspirin were given to the separate groups for 1 week. After 1 week, endothelial functions were reevaluated and aspirin resistance examined.

Echocardiography

Echocardiographic examination of the patients and the controls was performed with a Vivid 7 ultrasound machine (GE Medical Systems, Horten, Norway) using a broadband transducer. Evaluation of the patient group was performed at the time of entry into the study to detect the presence of end organ disease and to prevent the effect of antihypertensive treatment. The left ventricular end-diastolic dimension and wall thickness (interventricular septum and posterior wall) were measured from the parasternal long axis view according to the guidelines of the American Society of Echocardiography.²² The left ventricular mass (LVM) was calculated by the Devereux formula.²³ The left ventricular mass index (LVMI) was determined by dividing the LVM to body surface area. Left ventricular hypertrophy was defined as a LVMI > 125 g/m² in males and > 110 g/m² in females.¹⁸

A color-guided pulsed Doppler echocardiographic examination was performed from the apical 4-chamber view placing a sample volume at the tips of the mitral leaflets. Echocardiographic studies were performed under spontaneous respiration in all subjects. The E velocity, A velocity, E velocity deceleration time, isovolumetric relaxation time, and isovolumetric contraction time were measured, and the ratio of E/A was calculated. An E/A ratio < 1.0 was accepted as diastolic dysfunction.

Endothelial Function

Measurements of endothelial function were conducted in a temperature-controlled vascular research laboratory according to Celermajer's method.²⁴ Patients were evaluated in the morning, having abstained from alcohol, caffeine, and food for almost 8 hours and having reclined on the examination bed for 15 minutes before examination. All examinations were conducted by the 2 examiners blinded to the group and patient data. Arterial diameter was measured from high-resolution, 2-dimensional ultrasound images obtained with a Vivid 7 ultrasound machine (GE Medical Systems) with a 7.5 MHz linear array transducer. The right brachial artery was scanned over a longitudinal section 3 to 5 cm above the right elbow. The transmit (focus) zone was set to the depth of the anterior vessel wall, and depth and gain settings were optimized to identify the lumen-to-vessel wall interface. When a reasonable image was obtained, a mark was made on the skin for repeat examination; the arm was kept in the same position throughout the study.

Changes in diameter of the right brachial artery were measured at rest, during reactive hyperemia, again at rest, and after sublingual nitroglycerin (NTG) administration. A pneumatic tourniquet placed around the forearm distal to the target artery was inflated to a pressure of 250 mm Hg, and the pressure held for 5 minutes. After sudden cuff deflation, a second ultrasound scan was performed. Measurements were taken at 30, 60, and 90 seconds, and their average was calculated. An additional resting scan was recorded 15 minutes later to confirm the vessel recovery. Sublingual NTG spray (0.4 mg) was then administered, and the last scans were obtained 3 and 4 minutes later. Images were stored in digital format to enable offline analysis. The diameter of the brachial artery was measured from the anterior to the

posterior interface between the media and adventitia at a fixed distance. The mean diameter was calculated from 4 cardiac cycles synchronized with the R-wave peaks on the electrocardiogram. The diameter change caused by FMD was expressed as the percentage of change relative to that at the initial resting scan (% FMD). The diameter change caused by NTG administration was also expressed in the same way as the percentage change relative to that at the recovery scan (% NTG).

ASA Resistance

Venous blood samples were taken after 1 week of treatment. Aspirin resistance was evaluated with the platelet function analyzer (PFA-100; Dade Behring, Marbourg, Germany), which is a semiautomatic analyzer for determining platelet function that reproduces in vivo conditions.²⁵ For the analysis, citrated blood was passed through a capillary device, which stimulated in vivo conditions of shear stress. The PFA-100 gave a punctual lecture when blood flow stopped as a result of capillary occlusion that was due to platelet adhesion and subsequent aggregation to the exposure of platelet agonists that covered the membrane of a disposable cartridge. The final point in which blood flow stopped was defined as the closure time. One-type cartridges that utilized a membrane covered with epinephrine were used. Platelet function was altered when collagen/epinephrine closure time was prolonged. However, prolongation of the collagen/epinephrine closure time was observed only with the effect of aspirin. In our laboratory, normal epinephrine closure time ranged between 88 and 186 seconds. If the collagen/epinephrine closure time was less than 187 seconds with 2.1% analytical variance, it was interpreted as aspirin resistance or aspirin insensitivity.²⁶

Serum levels of Hs-CRP were measured with a rate nephelometric method (IMMAGE Immunochemistry System; Beckman Coulter Inc., Brea, CA). The measurement range in this method was 0.2 to 1440 mg/L, and the reference range was < 7.44 mg/L. Hemogram and all biochemistry measurements were carried out using standard methods.

Statistical Analysis

All analyses were performed using SPSS for Windows version 13 (SPSS, Inc., Chicago, IL). Normality tests were performed for all variables. Normally distributed continuous variables were analyzed with 2-tailed *t* test, and unequally distributed variables were compared by Mann-Whitney *U* test. Results were presented as mean $\pm$ standard deviation for continuous data or as percentages and numbers for categorical data. Clinical, echocardiographic, and endothelial findings of the patient and control groups were compared with a paired 2-sample *t* test, Mann-Whitney *U* test, or Student *t* test as appropriate. Categorical data and proportions were analyzed using a χ^2 test. A *P* value < 0.05 was accepted as statistical significance.

Intra- and Interobserver Variability

All echocardiographic studies and measurements were performed by the 2 cardiologist. The intraobserver variability was as follows: $r = 0.98$ for 2-dimensional and

M-mode echocardiographic measurements; $r = 0.97$ for Doppler measurements, and $r = 0.98$ for brachial artery measurements. Interobserver variability was as follows: $r = 0.97$ for echocardiographic measurements, $r = 0.96$ for Doppler measurements, and $r = 0.97$ for brachial artery measurements.

Results

Basic Characteristics

Fifty-eight hypertensive patients (mean age, 41 ± 7.6 years; 28 males) and 61 healthy subjects in the control group (mean age, 39 ± 4.7 years; 32 males) were included to the study. There was no significant difference between the age and gender of the groups. The systolic and diastolic BP of the patient group were higher compared to the control group ($P < 0.001$). The hematocrit percentages were significantly higher in the control group ($P < 0.001$) (table 1). Hemoglobin, white blood cells, and platelet counts were similar in both groups ($P =$ not significant). Blood glucose levels ($P < 0.001$), creatinine levels ($P < 0.009$), total cholesterol ($P < 0.001$), low-density lipoprotein (LDL) cholesterol levels ($P < 0.001$) were higher and high-density lipoprotein cholesterol levels were lower ($P = 0.001$) and statistically significant in patient group. In a similar manner, high sensitive C-reactive protein (Hs-CRP) levels were significantly higher in the hypertensive group ($P = 0.004$) (Table 1).

Echocardiographic Findings

Left ventricular wall thickness, left ventricular end-diastolic diameter, and muscle mass index were higher than the control group ($P < 0.001$). However, left ventricular hypertrophy was not detected in the patients and the control group. Left atrial diameter, ejection fraction, and fractional shortening were similar in both of groups ($P =$ not significant). Mitral E ($P = 0.003$) and A flow velocities ($P =$ not significant) were lower in the hypertensive group. Deceleration time was significantly longer ($P = 0.001$), and the E/A ratio was lower than the control group ($p = 0.001$); however, diastolic dysfunction was not detected in any of the patients (Table 2).

ASA Resistance

Frequency of ASA resistance was 20% and 26% in the control and hypertensive patient groups, respectively ($P =$ not significant). ASA resistance was similar in both 100 mg and 300 mg ASA users in the control group (20%) ($P =$ not significant). ASA resistance was 28% and 24% in 100 mg and 300 mg in hypertensive patients, respectively ($P =$ not significant) (Table 3).

Endothelial Dysfunction and ASA Treatment

There was no difference between the baseline brachial artery diameter and FMD-related brachial artery diameter in the control and patient groups before ASA treatment ($P =$ not significant). However, baseline FMD change percent in hypertensive patients was 9.8%, and it was significantly lower than the control group (12%)

Table 1. Comparison of Blood Pressure and Other Blood Tests in Control and Hypertensive Patients.

	Control	Hypertension	<i>P</i>
SBP	113 ± 8	165 ± 7	<0.001
DBP	75 ± 5	91 ± 2	<0.001
HR	82 ± 5	84 ± 7	NS
Hb	13.7 ± 1.7	13.5 ± 1.2	NS
Hct	40.6 ± 4.5	40.0 ± 3.0	<0.001
WBC	6870 ± 1690	6860 ± 1430	NS
Plt	240 ± 61	243 ± 44	NS
Glucose	85.6 ± 7.2	93.6 ± 5.8	<0.001
Urea	26.0 ± 7.0	27.7 ± 7.0	NS
Creatinine	0.78 ± 0.15	0.86 ± 0.16	0.009
Total cholesterol	157 ± 27	180 ± 13	<0.001
Triglyceride	94 ± 40	105 ± 29	NS
HDL	52 ± 14	49 ± 6	0.001
LDL	86 ± 22	120 ± 18	<0.001
Hs-CRP	3.95 ± 2.75	6.12 ± 1.43	0.004
CADPAGR	79.6 ± 14.2	78.2 ± 14.8	NS
CEPI AGR	160 ± 83	157 ± 83	NS

Abbreviations: CADPAGR, collagen ADP aggregation; CEPI AGR, collagen epinephrine aggregation; DBP, diastolic blood pressure; Hb, hemoglobin; Hct, hematocrit; HDL, high-density lipoprotein; HR, heart rate; Hs-CRP, high sensitive C-reactive protein; LDL, low-density lipoprotein; NS, not significant; Plt, platelet; SBP, systolic blood pressure; WBC, white blood cells.

($P < 0.001$). Similarly, nitroglycerin-induced dilatation (NID) change percent in hypertensive group was statistically lower in the control group ($P = 0.005$).

After treatment with ASA, there was no difference between the baseline brachial artery diameter and FMD diameter ($P = \text{not significant}$); however, both FMD ($P = 0.009$) and NID change percent ($P = 0.009$) were statistically lower than the control group. FMD change percent increased both in the control and hypertensive groups after ASA treatment, from 12.4% to 13.3% and 9.8% to 11.9%, respectively.

FMD diameter ($P < 0.001$) and FMD change percent ($P = 0.03$) were significantly improved in hypertensive patients after ASA treatment when groups were compared within each other before and after ASA treatment. Although there was a significant improvement in diameters after ASA treatment in the control group ($P < 0.001$), FMD change percent was not changed ($P = \text{not significant}$). NID diameters were increased after ASA treatment in both of groups ($P < 0.001$); however, there was no significant difference in NID change percent ($P = \text{not significant}$).

Brachial artery diameters ($P < 0.001$) and FMD change percent ($P = 0.022$, for 100 mg ASA; $P = 0.009$, for 300 mg ASA) was significantly increased both after 100 mg and

Table 2. Comparison of Echocardiographic Parameters

	Control	Hypertension	<i>P</i>
IVS (mm)	8.9 ± 0.9	9.4 ± 1.0	< 0.001
PW (mm)	8.7 ± 0.8	9.3 ± 0.9	< 0.001
LVEDD (mm)	46.8 ± 3.2	48.9 ± 3.4	0.003
LA (mm)	38 ± 3	39 ± 5	NS
LVMI (g/m ²)	88 ± 16	99 ± 19	<0.001
EF (%)	76 ± 5	74 ± 6	NS
FS (%)	42 ± 3	40 ± 4	NS
E (cm/s)	0.89 ± 0.16	0.74 ± 0.14	0.003
A (cm/s)	0.67 ± 0.11	0.65 ± 0.13	NS
E/A	1.32	1.13	0.001
DT (ms)	181 ± 16	198 ± 20	0.001
IVRT (ms)	88 ± 7	90 ± 7	NS

Abbreviations: A, late diastolic peak flow velocity; DT, deceleration time; E, early diastolic peak flow velocity; EF, ejection fraction; FS, fractional shortening; IVRT, isovolumetric relaxation time; IVS, interventricular septum; LA, left atrium; LVDD, left ventricular end-diastolic dimension; LVMI, left ventricular mass index; NS, not significant; PW, posterior wall.

300 mg ASA in hypertensive patients, when compared before and after 100 mg and 300 mg ASA within groups. NID change percent was not significant ($P = \text{not significant}$). All diameters were significantly increased after 100 and 300 mg ASA in the control group ($P < 0.001$); the increase in FMD and NID change percent was not significant ($P = \text{not significant}$) (Tables 4 and 5).

ASA Resistance vs Endothelial Dysfunction

Baseline diameter ($P = 0.04$, for ASA resistance [+]; $P < 0.001$, for ASA resistance [−]) and FMD diameter ($P = 0.043$, for ASA resistance [+]; $P < 0.001$, for ASA resistance [−]) were increased significantly irrespective of ASA resistance when endothelial functions were compared before and after ASA treatment in the ASA-resistant control group. Increase in FMD change percent was not statistically significant ($P = \text{not significant}$). Both diameters ($P = 0.002$, $P = 0.001$, for ASA resistance [+]; $P < 0.001$, for ASA resistance [−]) and FMD change percent were significantly increased in hypertensive patients irrespective of ASA resistance ($P = 0.02$, for ASA resistance [+]; $P < 0.012$, for ASA resistance [−]); however, the increase in NID change percent was not significant ($P = \text{not significant}$) (Table 6).

Discussion

In the literature, limited studies investigated the effects of aspirin on endothelium and aspirin resistance. The aim of our study was to investigate the effects of aspirin on endothelial functions in early stages of hypertension, to determine the prevalence of aspirin resistance in hypertensive patients who were not on medical treatment, and to investigate the relationship between endothelial functions and aspirin resistance.

Table 3. Prevalence of ASA Resistance in 100-mg and 300-mg Doses

	Control		Hypertension		Total	P
ASA resistance (+)	12 (20%)		15 (26%)		27 (23%)	NS
ASA resistance (–)	49 (80%)		43 (74%)		92 (77%)	NS
Total	61 (100%)		58 (100%)		119 (100%)	NS
	100 mg	300 mg	100 mg	300 mg		
ASA resistance (+)	6 (20%)	6 (19%)	8 (28%)	7 (24%)	27 (23%)	NS
ASA resistance (–)	24 (80%)	25 (81%)	21 (72%)	22 (76%)	92 (77%)	NS
Total	30 (100%)	31 (100%)	29 (100%)	29 (100%)	119 (100%)	NS

Abbreviations: ASA, acetylsalicylic acid; NS, not significant.

Table 4. Comparison of the Endothelial Functions in the Control Group and Hypertensive Patients Before and After Treatment

	Control	Hypertension	P
Basal BA diameter	38.6 ± 5.0	38.5 ± 4.9	NS
Basal FMD diameter	43.3 ± 4.9	42.2 ± 4.7	NS
Basal FMD %	12.4 ± 3.7	9.8 ± 3.0	< 0.001
Basal NID diameter	43.6 ± 5.0	42.9 ± 4.7	NS
Basal NID %	13.3 ± 3.3	11.6 ± 3.7	0.005
ASA diameter	40.1 ± 5.0	39.8 ± 4.6	NS
ASA FMD diameter	45.4 ± 5.2	44.1 ± 4.4	NS
ASA FMD %	13.3 ± 5.0	11.0 ± 3.1	0.009
ASA NID diameter	46.0 ± 5.3	44.7 ± 4.4	NS
ASA NID %	13.5 ± 5.0	11.9 ± 3.1	0.009

Abbreviations: ASA, acetylsalicylic acid; BA, brachial artery; FMD, flow-mediated dilatation; NID, nitroglycerin-induced dilatation; NS, not significant.

The vascular endothelium plays a key role in the regulation of cardiovascular homeostasis and in the control of BP.^{1,2} Systemic inflammation and endothelial activation are implicated in the development of hypertension.¹⁹

Hs-CRP, which is an inflammation marker, was found statistically high as the other studies demonstrated. Gupta et al demonstrated that Hs-CRP increased in all stages of hypertension; however, they found no significant difference between stage 1 and 2 hypertension.²⁷ In our study, Hs-CRP levels were in normal range both in hypertensive patients and the control group; however, it was significantly higher in hypertensive patients. The slightly increased C-reactive protein (CRP) levels may result from exclusion of all other possible causes that may increase CRP levels and inclusion of newly diagnosed uncomplicated patients. The Hs-CRP levels may increase in further stages of hypertension and with the development of complications. This supports that inflammation may play role in the etiology of hypertension.

The frequency of aspirin resistance in clinical studies might have been affected by the diagnostic method of aspirin resistance and the dose of aspirin studied. Because of the lack of a gold standard method for diagnosis, different

laboratory parameters for the detection of aspirin resistance were used in the previous studies.²¹ Therefore, although the PFA-100 may be useful in identifying patients with high platelet reactivity (with or without aspirin therapy), its specificity for aspirin resistance was uncertain.²⁸ Gum et al investigated the effect of different diagnostic methods on the prevalence of aspirin resistance and reported a prevalence of 9.5% with the PFA-100 device and 6% with the optical platelet aggregation test in stable coronary artery disease patients who received 100 mg/day of aspirin.²⁹ The frequency of aspirin resistance detected with the PFA-100 method was reported as 9.5% to 52% in patients with stable coronary artery disease, 10% in patients with peripheral artery disease, 21.5% in patients with diabetes mellitus, and 21.9% in patients with metabolic syndrome.^{30–32}

Previous studies on the effects of different doses of ASA on different populations showed that increasing ASA dose decreased the frequency of ASA resistance both in healthy subjects and in patients with stable coronary artery disease.^{33,34} Gonzales-Conejero et al reported that 33.3% of healthy subjects under the treatment of 100 mg ASA had resistance according to the PFA-100 device, and none of the subjects displayed ASA resistance when the dose was increased up to 500 mg/day.³³ Lee et al found that the efficacy of ASA in patients with stable coronary artery disease was 68% when the ASA dose was 100 mg or lower, 83.3% when the dose was 150 mg, and 100% when the dose was 300 mg.³⁴

ASA resistance of healthy individuals in our study population was correlated with the literature. We have not detected a difference in ASA resistance between 100 mg and 300 mg ASA. ASA resistance frequency in hypertensive patients was decreased as the dose increased; however, it was statistically insignificant because of the low patient number.

Literature review of other factors that could be related to ASA resistance included female gender, age, smoking, hypertension, total and LDL cholesterol levels, diabetes mellitus, increased platelet turnover, use of ibuprofen, obesity, coronary artery disease, and exercise.^{21,29,35,36} Age and sex distribution in our study population was similar. Only total and LDL cholesterol levels and fasting blood glucose level were higher than normal in the hypertensive patient group; however, there were no diabetic patients.

Table 5. Comparison of the Endothelial Functions in the Control Group and Hypertensive Patients and Between the Groups Before and After Treatment

	Control			Hypertension		
	Pre-ASA	Post-ASA	P	Pre-ASA	Post-ASA	P
BA diameter	38.6 ± 5.0	40.1 ± 5.0	< 0.001	38.5 ± 4.9	39.8 ± 4.6	< 0.001
FMD diameter	43.3 ± 4.9	45.4 ± 5.2	< 0.001	42.2 ± 4.7	44.1 ± 4.4	< 0.001
FMD %	12.4 ± 3.7	13.3 ± 5.0	NS	9.8 ± 3.0	11.0 ± 3.1	0.03
NID diameter	43.6 ± 5.0	46.0 ± 5.3	< 0.001	42.9 ± 4.7	44.7 ± 4.4	< 0.001
NID %	13.3 ± 3.3	13.5 ± 5.0	NS	11.6 ± 3.7	11.9 ± 3.1	NS
Control						
ASA 100 mg			ASA 300 mg			
	Pre-ASA	Post-ASA	P	Pre-ASA	Post-ASA	P
BA diameter	38.0 ± 4.8	39.5 ± 5.0	< 0.001	39.2 ± 5.3	40.8 ± 4.9	< 0.001
FMD diameter	42.6 ± 4.8	44.5 ± 5.0	< 0.001	43.9 ± 5.1	46.3 ± 5.2	< 0.001
FMD %	12.4 ± 4.2	12.9 ± 4.9	NS	12.8 ± 3.2	13.5 ± 5.1	NS
NID diameter	42.8 ± 4.9	45.1 ± 5.3	< 0.001	44.5 ± 5.2	46.9 ± 5.2	< 0.001
NID %	12.6 ± 3.5	12.9 ± 4.9	NS	13.2 ± 3.2	13.7 ± 5.1	NS
Hypertension						
ASA 100 mg			ASA 300 mg			
	Pre-ASA	Post-ASA	P	Pre-ASA	Post-ASA	P
BA diameter	37.9 ± 5.0	39.3 ± 4.6	< 0.001	39.0 ± 4.8	40.3 ± 4.6	0.001
FMD diameter	41.7 ± 5.0	43.6 ± 4.6	< 0.001	42.6 ± 4.5	44.6 ± 4.3	< 0.001
FMD %	10.2 ± 2.5	11.1 ± 3.0	0.022	9.4 ± 3.5	10.9 ± 3.3	0.009
NID diameter	42.5 ± 4.9	44.2 ± 4.6	< 0.001	43.3 ± 4.5	45.1 ± 4.3	0.001
NID %	11.8 ± 3.4	12.3 ± 3.0	NS	11.3 ± 4.0	11.6 ± 3.8	NS

Abbreviations: ASA, acetylsalicylic acid; BA, brachial artery; FMD, flow-mediated dilatation; NID, nitroglycerin-induced dilatation; NS, not significant.

Endothelial functions, which measured above brachial arteries, were significantly impaired in hypertensive patients. Although there is no cutoff value for FMD change percent, changes ≤10% are considered as endothelial dysfunction. The cutoff value for hypertensive patients and controls were 9.8% and 12.4%, respectively. FMD change percent in the hypertensive patient group was lower than 10%. The reason for not being very low may relate to our hypertensive group, which included newly diagnosed patients without complications and an advanced stage of hypertension.

After ASA treatment, FMD change percent increased in the control group and hypertensive patients from 12.4% to 13.4% and 9.8% and 11.8%, respectively. FMD change percent after ASA treatment was significant between the 2 groups.

Several prospective studies have demonstrated that endothelial dysfunction is associated with a high incidence of cardiovascular events.^{6,7,12,13} A significant correlation between endothelial dysfunction and hypertension,¹⁴ coronary artery disease (CAD),³⁷ diabetes mellitus,³⁸ or erectile dysfunction has been demonstrated previously.³⁹ In

hypertensive patients, basal NO activity is decreased and endothelium-dependent vasodilation in response to acetylcholine is impaired in forearm circulation.^{9–11} Furthermore, abnormal endothelial function has been reported in patients with chronic heart failure.^{40,41}

This suggests that a reduction in NO release from the vascular and endocardial endothelium is involved in impaired vascular and myocardial relaxation.⁴⁰ Although diastolic dysfunction was not detected in both of the groups, mitral flow E/A ratio was significantly lower when compared to the control group; similarly, vascular endothelial dysfunction was significant in patients with hypertension. This finding suggests that vascular endothelial functions and diastolic functions develop at the same time or consecutively.

Endothelial functions were improved in hypertensive patients after treatment with both ASA doses; however, the improvement in the control group was not significant. FMD change percent were significantly increased in hypertensive patients irrespective of ASA resistance. As the endothelial functions were normal in the control group, it is expected that ASA treatment would not affect endothelial functions.

Table 6. Comparison of Endothelial Functions of ASA-Resistant and Nonresistant Patients Before and After Treatment

Control						
ASA Resistance (+)			ASA Resistance (–)			
	Pre-ASA	Post-ASA	P	Pre-ASA	Post-ASA	P
BA diameter	37.9 ± 4.4	38.9 ± 3.9	0.04	38.8 ± 5.2	40.4 ± 5.2	< 0.001
FMD diameter	41.3 ± 4.3	42.4 ± 3.6	0.043	43.7 ± 5.0	46.0 ± 5.3	< 0.001
FMD %	9.0 ± 3.7	9.6 ± 4.0	NS	12.9 ± 3.5	13.4 ± 4.6	NS
NID diameter	41.5 ± 4.2	42.6 ± 4.0	0.08	44.0 ± 5.2	46.6 ± 5.4	< 0.001
NID %	10.2 ± 4.0	10.6 ± 4.0	NS	13.8 ± 3.0	14.3 ± 4.6	NS
Hypertension						
ASA Resistance (+)			ASA Resistance (–)			
	Pre-ASA	Post-ASA	P	Pre-ASA	Post-ASA	P
BA diameter	38.8 ± 4.7	40.2 ± 4.2	0.002	38.3 ± 5.0	40.0 ± 4.7	< 0.001
FMD diameter	42.0 ± 4.6	42.8 ± 4.4	0.001	42.2 ± 4.8	44.5 ± 4.4	< 0.001
FMD %	8.4 ± 1.8	9.9 ± 2.6	0.02	10.2 ± 3.3	11.6 ± 3.1	0.012
NID diameter	42.5 ± 4.6	43.4 ± 4.6	0.003	43.0 ± 4.7	45.2 ± 4.3	< 0.001
NID %	9.4 ± 2.1	9.8 ± 2.6	NS	11.7 ± 3.7	12.2 ± 3.2	NS

Abbreviations: ASA, acetylsalicylic acid; BA, brachial artery; FMD, flow-mediated dilatation; NID, nitroglycerin-induced dilatation; NS, not significant.

ASA treatment improved the endothelial functions in hypertensive patients as their endothelial functions were impaired.

The benefit of antiplatelet therapy with aspirin in patients with hypertension is well established. It is suggested that aspirin modulates acetylcholine-induced peripheral vasodilation in patients with atherosclerosis, possibly via inhibition of 1 or more cyclooxygenase-dependent vasoconstrictors. A study investigating the effect of 1 dose specific thromboxane A₂-receptor antagonist (S18886) on endothelial dysfunction in coronary artery disease found that ASA treatment was related to FMD and acetylcholine-induced vasodilatation.⁴² This shows us that high doses of ASA were not necessary to improve endothelial functions. This dose of ASA also improved endothelial functions, and ASA taking time was important.⁴² This finding suggests that ASA effects endothelial functions with a mechanism other than antiaggregant effect.

Oxidative stress and endothelial dysfunction are consistently observed in hypertensive patients; however, emerging evidence suggests that they also have a causal role in the molecular processes leading to hypertension. ASA is a potent antioxidant agent, and reduction in vascular production of superoxide in normotensive and hypertensive rats has been demonstrated in previous studies.⁴³ Hermida et al showed that low-dose ASA (100 mg) had a favorable effect on blood pressure of patients in a study consisting of 244 prehypertension patients.⁴⁴ Recent studies have also demonstrated that ASA induces NO release from vascular endothelium. This effect appears to be due to a direct acetylation of the endothelial NO synthase protein.⁴⁴ Systemic inflammation impairs endothelium-dependent dilatation in

humans and provides direct evidence that pretreatment with an anti-inflammatory dose of aspirin protects from this endothelial dysfunction. This vasculoprotective effect of aspirin does not seem to be attributable to inhibition of vascular constrictor prostanoid synthesis, because locally infused aspirin did not restore endothelial function in vaccinated subjects. The abrogation of the cytokine response to inflammatory stimuli provides a novel mechanism by which aspirin can modulate endothelial function.²⁰ Favorable effects of ASA usage on endothelium even in low doses in hypertensive patients shows the importance of ASA usage in hypertensive patients. Specifically, the present study raises the possibility that protective effects of aspirin on endothelial functions may contribute to the efficacy of this drug in reducing cardiovascular risk in certain situations.²⁰

We detected improvement in endothelial functions with ASA treatment irrespective of ASA resistance. The frequency of ASA resistance was 26% in the hypertension patient group. Aspirin resistance was 21% in a study investigating the prevalence of aspirin resistance in hypertension.⁴⁵ The prevalence was 17.8% and 25.6% in normotensive and hypertensive patients, respectively. These patients were on treatment; however, all of our patients were newly diagnosed and were not on treatment. In a recent study, Feher et al showed that ASA resistance was lower in hypertensive patients who were on ACE inhibitor and β -blocker treatment. These drugs may exert an additive antiplatelet action when combined with aspirin.⁴⁶

Such a high prevalence of aspirin resistance may be anticipated in hypertensive patients, because a number of factors, including increased arterial stiffness, shear stress, and endothelial dysfunction, might contribute to altered

platelet reactivity and lead to a relatively high frequency of aspirin resistance among subjects with hypertension.³²

The relationship between aspirin resistance and inflammation remains unclear. However, both aspirin resistance and increased inflammation markers are found more frequently after acute coronary events or in chronic diseases such as diabetes mellitus. Residual platelet activity as a result of inflammation that is not fully inhibited by aspirin may be responsible for aspirin resistance. However, there is no direct evidence that aspirin resistance is due to increased inflammatory mediators per se.²⁸

ASA resistance is related to thrombocyte functions. Therefore, the favorable effects of ASA treatment on endothelial functions is seen irrespective of ASA resistance. These favorable effects increase with increased doses. Also, decreased ASA resistance with increased doses attenuates this effect.

In the case of hypertension, this is likely not only related to altered production of thromboxane A₂, but also given the pleiotropic function of aspirin, may be related to the activation of NO synthase and an increase in NO production by platelets. The increased NO may then counteract various prothrombotic and hypertensive factors, and thereby may be implicated in the clinical efficacy of aspirin. Cheng and coworkers investigated the relationship between endothelial dysfunction and aspirin resistance in 54 patient with stable CAD and reported that endothelial dysfunction is a significant factor in aspirin resistance.⁴⁷

Various studies have shown that NO synthase decreased as the endothelial functions deteriorated. This may decrease ASA's effect on NO or ASA resistance and may result in endothelial dysfunction as the other studies revealed.

Diabetes, atherosclerosis, hyperlipidemia, and smoking, which are known risk factors for endothelial dysfunction, were not included in this study. Also, the patient group was not on medication. Our data helped us to understand the effect of ASA on endothelium more clearly than other studies. Our study showed that endothelial dysfunction in early stages of hypertension does not result in the development of ASA resistance.

Limitations

One of the limitations of our study is the relatively low number of patients. ASA resistance was lowest in the patient group receiving 300 mg ASA; however, it was statistically not significant. Another limitation was that we could provide information only for Hs-CRP among inflammation parameters; however, the aim of our study was not to show inflammation. Also, there is no gold standard to evaluate ASA resistance, and therefore we investigated ASA resistance with the PFA-100 test. We have not compared other evaluation techniques. We also could not evaluate endothelial functions in early and late stages of hypertension and ASA resistance in complicated advanced-stage hypertensive patients. We do not have data on the long-term effects of ASA treatment on endothelial function.

Conclusion

Impairment in endothelial function begins in the early stages of the disease and impairment in diastolic function

parameters frequently coexist. The prevalence of ASA resistance differs according to the test and patient groups. We found the prevalence of ASA resistance in the study group and control group between 26% and 20%, respectively. Also, we do not find any difference in ASA resistance between the 2 different ASA doses. Endothelial functions were improved with all ASA doses in hypertensive patients irrespective of ASA resistance. Further studies are needed to evaluate the effects of ASA on endothelial functions in early and advanced stages of hypertension.

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