

## EFFECTS OF SMOKELESS TOBACCO “MARAS POWDER” USE ON MARKERS OF ENDOTHELIAL DYSFUNCTION

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### ABSTRACT

**Aim:** To compare brachial artery flow-mediated dilation (FMD) in subjects who use smokeless tobacco, smoke cigarettes, or do not use any tobacco product.

**Materials and methods:** In the study, participants were 35 Maras powder users, 36 cigarette smokers and 30 nontobacco user subjects. Blood samples were collected and hematological parameters and lipid parameters were measured. Homocystein and hsCRP levels were measured in serum and compared between the groups. Flow-mediated dilation was assessed using brachial artery duplex ultrasonography.

**Results:** Plasma total cholesterol, LDL-cholesterol, triglyceride levels were significantly higher in Maras powder and cigarette smokers group than in the nontobacco user group ( $p < 0.001$ ). Plasma HDL-cholesterol levels were significantly lower in Maras powder and cigarette smokers group than in the nontobacco user group ( $p < 0.001$ ). Serum homocystein levels and hsCRP levels were found significantly higher in Maras powder and cigarette smokers group compared to the nontobacco user group ( $p < 0.001$ ) whereas there was no significant difference between the Maras powder and cigarette smokers group. Baseline brachial artery diameter, endothelium-dependent FMD induced by reactive hyperemia, and endothelium-independent dilation induced by administration of sublingual nitroglycerin were measured. Brachial artery endothelium-dependent FMD was found significantly lower in Maras powder ( $5,73 \pm 2,99$ ) and cigarette smokers ( $6,55 \pm 3,50$ ) group compared to the nontobacco users ( $11,86 \pm 4,61$ ) ( $p < 0.001$ ,  $p < 0.001$ , respectively).

**Conclusions:** Brachial artery FMD, a surrogate for endothelial dysfunction, was significantly impaired in smokeless tobacco users and cigarette smokers compared with nonusers of tobacco.

**Key words:** Maras powder, smokeless tobacco, flow-mediated dilatation, endothelial function, brachial artery.

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### Introduction

There are various forms of tobacco use, which can be classified such as smoking or smokeless tobacco (ST). ST is a different form of tobacco use. ST is widely used as chewing tobacco and as oral snuff in the United States, Western Europe, southern parts of the Kingdom of Saudi Arabia, southern African countries and the Sudan in northeast Africa<sup>(1-4)</sup>. Also, in Kahramanmaraş, a city located in southern Turkey, ST is widely consumed instead of cigarette smoking and this habit has become increasingly popular among the males, especially among children and male adolescents.

Snuff is a term used to describe a wide variety of products containing finely ground tobacco as a

principal constituent and other additives. Snuff is either inhaled to the nasal cavity or dipped in the oral cavity<sup>(1)</sup>. According to preparation methods snuff is called differentially in various regions of the world. In Kahramanmaraş, a different type of ST, locally called as “Maras powder (MP)” or “oral powder” and also “powder” has been used for a long time. This powder is mostly preferred while trying to quit smoking or lessen it. Tobacco used for manufacture of MP is of the species *Nicotiana rustica* Linn (NRL). The leaves of a plant known as “crazy tobacco” locally are powdered and this powder is mixed with the ash of wood especially oak, walnut or grapevine. First of all, sun-dried leaves of this plant are powdered and mixed with the ash in approximately 1:2 or 1:3 proportions (tobacco and

oak, resp.). Then, water is sprinkled onto this mixture for humidification. A small amount of this mixture, sometimes as portion-bag-packed (approximately 1g a quid) is applied between the lower labial mucosa and gingival for 4-5 minutes and even as long as 1-2 h. This region of the mouth has many capillary vessels; therefore, nicotine is quickly absorbed into circulation. This procedure is repeated many times during the day<sup>(2,3)</sup>.

Smoking causes endothelial dysfunction (ED) through impairment of nitric oxide (NO) production or increased oxidative stress by a large number of free radicals known to exist in smoke<sup>(5)</sup>. Since endothelial dysfunction is a well-documented early phenomenon in atherosclerosis as it precedes structural changes and clinical manifestations, major research efforts have focused on the detection of endothelial dysfunction in humans<sup>(6)</sup>. In addition, a number of studies and meta-analysis have shown that smokeless tobacco had an increased risk for cardiovascular diseases<sup>(7-10)</sup>. Laboratory and experimental evidence indicate that atherosclerosis, in addition to being a disease of lipid accumulation, also represents a chronic inflammatory process<sup>(11)</sup>. Thus, researchers have hypothesized that inflammatory markers such as high-sensitivity C-reactive protein (HSCRP) and homocysteine may provide an adjunctive method for global assessment of cardiovascular risk<sup>(12,13)</sup>.

A noninvasive test to evaluate extent of ED, flow-mediated dilatation (FMD) of the brachial artery (BA) by ultrasonography, has been used in patients with several coronary risk factors, coronary artery disease, peripheral arterial disease, stroke and is an independent predictor of cardiovascular events<sup>(14,15)</sup>. The aim of this study was to evaluate whether there is difference between the effects of Maras powder and cigarette smoking for the presence of ED by measuring known parameters of ED such as the lipid profile, homocysteine and high-sensitivity CRP (hsCRP), as well FMD. Therefore, we compared the vascular endothelial function by using ultrasonographic assessment of flow-mediated dilatation (FMD) on the brachial artery in Maras Powder users, cigarette smokers, and nonusers of tobacco.

## Methods

### *Study Populations*

This is a prospective, double-centered study, conducted in Department of Chest Diseases,

Kahramanmaraş Sutcu Imam University and Department of Cardiology, Necip Fazıl Sehir State Hospital between January 2014 and March 2014. Subjects having cigarette and Maras powder were collected from chest disease clinic of Kahramanmaraş Sutcu Imam University, Faculty of Medicine. History was taken and physical examinations were done. Selection criteria of the individuals were as follows: Cigarette smokers have been smoking one pack of cigarettes for at least one years, Maras powder users have been using it at least one packs for at least one years and age- and sex-matched nontobacco users for control.

Patients who use Maras powder and cigarette together were not included in the study. Subject's age, duration and frequency of use of Maras powder, duration and package- year of cigarette smoking were all recorded. Exclusion criteria were: patients with hypertension (systolic >140 mmHg/diastolic 90 mmHg), diabetes, obesity, congestive heart failure, chronic obstructive lung disease, malignancies, renal failure, ischemic heart disease, peripheral vascular disease, gastrointestinal disease, systemic illness and history of taking medications for the above-mentioned diseases and history of alcohol consumption. Finally, 101 patients (35 Maras powder users, 36 cigarette smokers and 30 nontobacco users) fulfilling the inclusion criteria were selected for participation and completed the study. The study was in accordance with the Second Declaration of Helsinki and was approved by Kahramanmaraş Sutcu Imam University Ethics Committee and all subjects gave their informed consent.

### *Laboratory Analyses*

Blood samples were taken from antecubital veins in the sitting position, after a minimum 8 hour fast, between 08:00 and 10:00 AM. Blood samples were then centrifuged at room temperature for 10 min. Separated plasma and serum were stored at -80°C until analysis. Serum levels of high-density lipoprotein cholesterol (HDL-C), LDL cholesterol (LDL-C) and triglyceride were measured with auto-analyzer kits (Roche, Germany) using an auto-analyzer (Roche/Hitachi modular P800). High-sensitivity CRP levels were evaluated using a commercial ELISA kit (DRG International Inc, USA). Serum homocysteine levels were measured using a high-pressure liquid chromatography method (Shimadzu device; Immuchrome, Germany)

### Measurements

Blood pressure was measured with sphygmomanometer on both right and left arms while the patients were sitting after 10 minutes of resting. Measurements were made 3 times and the median of 3 measurements was obtained. Body weight was measured to the nearest 0.5 kg in light clothing without shoes. Height was measured to the nearest 0.5 cm. Body mass index (BMI) was calculated as kilograms divided by square meters (kg/m<sup>2</sup>).

### Evaluation of brachial artery FMD

All measurements of brachial FMD were performed by a designated cardiologist with experience using a Mitsubishi ultrasound system machine with a 12L probe at the radio-diagnosis department. Patients were asked to avoid strenuous exercise just before FMD measurement and also to abstain from consuming alcohol or caffeinated drinks for at least 12h before being tested. Ultrasonographic examinations were carried out in a quiet environment at a temperature of 21°C to 23°C, after a fasting period of 8 to 12h, systolic blood pressure (SBP) and diastolic blood pressure (DBP) of all patients were measured after a resting period of 10 minutes, after which they were asked to lay in a comfortable supine position. The right BA is first palpated just above the antecubital fossa after which the transducer probe is placed longitudinally. The BA is then screened for a segment with no tortuosity to allow for an accurate measurement of FMD and 2-dimensional (2D) images in which the front and back intimal surfaces between the lumen and the blood vessel wall can be clearly detected are evaluated for best image. Using the enlarging and focusing features of the device, 3 consecutive intima-to-intima measurements of BA diameter are made, the average of which is recorded as the basal diameter. After basal measurements were recorded, a sphygmomanometer cuff is placed on the upper arm, proximal to the right antecubital fossa to generate a flow stimulus in BA. Great care is taken to ensure subsequent measurements are made at the end of diastole using simultaneous electrocardiographic monitoring. Cuff is inflated to a 50 mmHg higher value than the patient's SBP for complete blockage of antegrade arterial flow allowing development of ischemia, and this pressure is maintained for 5 minutes. Sixty seconds after removal of cuff, longitudinal 2D images are obtained. Once again, the average of 3 different measurements of BA lumen diameter is recorded as the endothelium-

dependent vasodilator response (EDVR). Flow-mediated dilatation is expressed as a percentage increase in lumen diameter from the baseline using the Formula  $FMD = [(EDVR - BD) / BD] \times 100$  (14).

### Results

Maras powder group was consisted of 19 men (63,3%) and 11 women (36,7%) (mean age: 51,73±10,46years), cigarette smokers group was consisted of 20 men (64,5%) and 11 women (35,5%) (mean age: 57,35±8,44 years) and the nontobacco users group was consisted of 21 men (63,6%) and 12 women (36,4%) (mean age: 58,67±9,26 years). There was no difference between groups according to age, gender, and BMI. Maras powder and cigarette smoking durations were similar (Table1).

	Maras Powder (n=30)	Cigarette Smokers (n=31)	Nontobacco users (n=33)	P
Gender (F/M)	36,7/63,3	35,5/64,5	36,4/63,6	0,995
Age, years	51,73±10,46 *	57,35±8,44	58,67±9,26	0,011
BMI, kg/m <sup>2</sup>	29,31±3,96 **	26,24±4,72	28,47±4,44	0,022
Tobacco use duration (year)	11,26 ± 2,03	13,0 ± 3,86	-	0,176

**Table 1:** General Characteristics of Study Population.

Abbreviations: BMI, body mass index; F, female; M, male

\* $p < 0.05$  for comparison with non tobacco users.  $p: 0,012$

\*\* $p < 0.05$  for comparison with cigarette smokers.  $p: 0,021$

When plasma lipid levels of all groups were compared, it was seen that there was a significant difference between the tobacco users (Maras powder and cigarette smokers) and nontobacco users group. Plasma total cholesterol, LDL cholesterol, triglyceride levels were significantly higher in Maras powder group than in the nontobacco users group whereas there was no significant difference between the Maras powder and cigarette smokers group. Plasma HDL-cholesterol levels were significantly lower in Maras powder and cigarette smokers group than in the nontobacco users group (Table 2).

Blood pressure was higher in the Maras powder and cigarette smokers group compared to the nontobacco users group ( $p < 0.001$ ). Plasma hematological parameters were compared. The platelet counts and mean platelet volumes were found higher in Maras powder group than in the nontobacco users group. There were no statistically significant differences in hematological parameters between patients with Maras powder and cigarette smokers group (Table 2).

	Maras Powder (n=30)	Cigarette Smokers (n=31)	Nontobacco users (n=33)	p
Systolic BP (mmHg)	134,00 ± 11,99	128,55 ± 9,055	118,33 ± 7,36	<b>0,001</b>
Diastolic BP (mmHg)	83,66 ± 8,29	79,03 ± 7,68	76,21 ± 6,49	<b>0,001</b>
Glucose (mg/dl)	106,93± 33,38	102,58±16,0	98,52±13,06	0,401
Total cholesterol (mg/dl)	209,83 ± 20,87	213,29 ± 18,51	186,39 ±8,41	<b>0,001</b>
Triglycerides (mg/dl)	198,37± 78,74	167,10 ± 46,71	119,70 ± 16,27	<b>0,001</b>
HDL cholesterol (mg/dl)	37,64 ± 6,48	37,19 ± 2,84	48,67 ± 8,85	<b>0,001</b>
LDL cholesterol (mg/dl)	141,91± 25,65	133,42 ± 28,38	106,73 ± 6,69	<b>0,001</b>
Hemoglobin (g/dl)	14,95± 1,52	14,071±1,50	13,00±1,63	0,01
Hematocrit (%)	42,73 ± 1,31	42,64 ± 1,22	42,42 ± 1,25	0,732
Platelets counts (x10 9l)	349,2 ± 96,36	344,61± 93,24	202,87 ± 71,33	<b>0,001</b>
Mean platelet volume	8,75± 0,94	8,57 ± 0,954	7,07 ± 0,673	<b>0,001</b>

**Table 2:** Comparison of Blood Pressures and Laboratory Characteristics Between Groups.

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, BP: Blood pressure

Serum homocystein levels were found significantly higher in Maras powder (7,6±1,4µmol/l) and cigarette smokers (7,4±1,6µmol/l) group compared to the nontobacco users group (2,5±0,7µmol/l) (p<0.001, p<0.001, respectively) whereas there was no significant difference between the Maras powder and cigarette smokers group (Table 3).

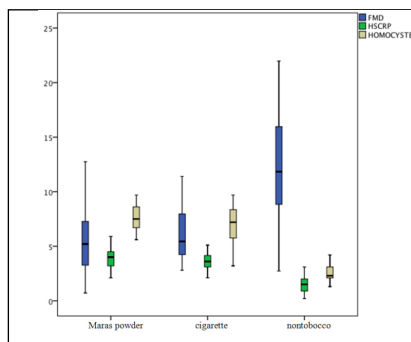
	Maras Powder (n=30)	Cigarette Smokers (n=31)	Nontobacco users (n=33)	p
hsCRP, mg/dL	3,95 ± 0,9	3,56 ± 0,76	1,43 ± 0,76 *	<b>0,001</b>
Homocysteine, mmol/L	7,66±1,49	7,04±1,68	2,55±071	<b>0,001</b>
FMD, %	5,73 ±2,99	6,55 ±3,50	11,86 ±4,61 **	<b>0,001</b>

**Table 3:** Comparison of hsCRP, Homocysteine Levels and FMD Between Groups.

hsCRP, high-sensitivityC-reactive protein; FMD, flow-mediated dilatation.

\*p<0.05 for comparison with maras powder and cigarette smokers p:0,001

\*\*p<0.05 for comparison with maras powder and cigarette smokers p:0,001



**Fig. 1:** Comparison of FMD, hsCRP and homocystein levels among the Maras powder, cigarette smokers, nontobacco users.

Serum levels of hsCRP were found significantly higher in Maras powder (3,95±0,9 µmol/l) and cigarette smokers (3,56±0,76 µmol/l) group compared to the nontobacco users group (1,43±0,7 µmol/l) (p<0.001, p<0.001, respectively) whereas FMD measurements were significantly lower (p<0.001) (Table3) (Figure 1).

Brachial artery endothelium-dependent FMD was found significantly lower in Maras powder (5,73 ± 2,99) and cigarette smokers (6,55 ± 3,50 ) group compared to the nontobacco users (11,86 ± 4,61 )(p<0.001, p<0.001, respectively). The FMD was not statistically different between smokeless tobacco users and cigarette smokers (Table 4). Endothelium-independent vasodilation induced by nitroglycerin was found significantly lower in Maras powder (10,83 ± 4,16) and cigarette smokers (12,34 ± 4,93 ) group compared to the nontobacco users (19,03 ± 4,99) (p<0.001, p<0.001, respectively) whereas there was no significant difference between the Maras powder and cigarette smokers group (Table 4).

	Maras Powder (n=30)	Cigarette Smokers (n=31)	Nontobacco users (n=33)	p
Brachial artery diameter at baseline (mm) (D1)	4,093 ± 0,247	4,110± 0,268	3,88 ± 0,33*	<b>0,001</b>
Endothelial-dependent flow-mediated dilation (%) (FMD)	5,73 ± 2,99	6,55 ± 3,50	11,86 ± 4,61	<b>0,001</b>
Endothelial-independent NTG-induced vasodilation (%) (D2)	10,83 ± 4,16	12,34 ± 4,93	19,03 ± 4,99**	<b>0,001</b>

**Table 4:** Comparison of D1, D2, FMD.

D1, baseline diameter of brachial artery; D2, hyperemia-induced diameter; FMD - flow-mediated vasodilation

\*p<0.05 for comparison with maras powder and cigarette smokers p:0,001

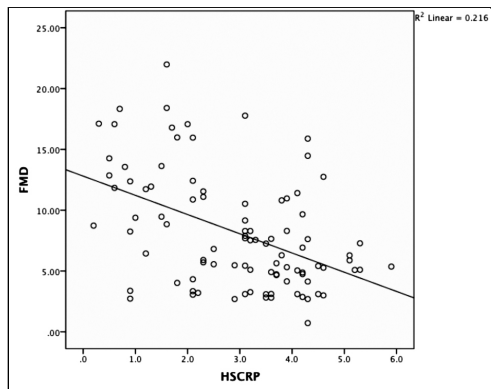
\*\*p<0.05 for comparison with maras powder and cigarette smokers p:0,001

Results of correlation analysis of variables likely to be associated with FMD are presented in Table 5. There was a significant negative correlation between FMD and BMI (r=-0.67, p=0.519), systolic BP (r=-0.343, p<0.001), diastolic BP (r=-0.252, p=0.014), plasma total cholesterol (r=-0.329, p<0.001), LDL-cholesterol (r=-0.354, p<0.001), triglyceride (r=-0.373, p<0.001), hsCRP(r=-0.432, p<0.001), homocystein (r=-0.490, p<0.001) levels in all patients.

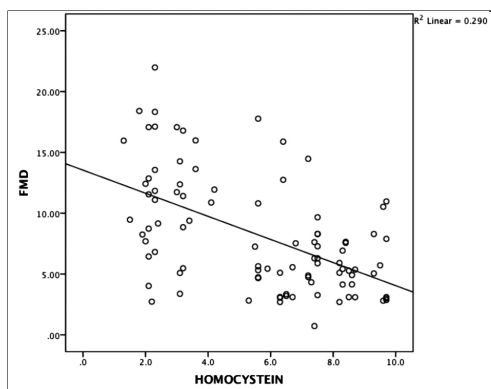
In addition, there was a significant positive correlation between FMD and HDL cholesterol (r=0.410, p<0.001) (Figure 2,3).

variable	FMD	
	r	P value
BMI	-0.67	0.519
Systolic BP (mmHg)	-0.343	0.001
Diastolic BP (mmHg)	-0.252	0.014
Total cholesterol (mg/dl)	-0.329	0.001
LDL cholesterol (mg/dl)	-0.354	0.001
HDL cholesterol (mg/dl)	0.41	0.001
Triglycerides (mg/dl)	-0.373	0.001
hsCRP	-0.432	0.001
homocystein	-0.49	0.001

**Table 5:** Results of Spearman correlation analysis on all patients included in the study.



**Fig. 2:** Significant negative correlation between FMD and hsCRP ( $r=0.432$ ,  $p<0.001$ ).



**Fig. 3:** Significant negative correlation between FMD and homocystein. ( $r=0.490$ ,  $p<0.001$ ).

## Discussion

It has been reported that Maras powder use increases oxidative stress and thus quickens atherosclerosis in addition to its negative effects on many tissues and organ systems<sup>(16-20)</sup>. Measurement of FMD provides a noninvasive assessment of the

functional capacity of the vascular endothelium. The endothelium plays a central role in the modulation of vascular tone, the inhibition of platelet aggregation and vascular smooth muscle proliferation and a key participation in angiogenesis under appropriate conditions. NO is well recognized as playing a pivotal role in these endothelial properties<sup>(21)</sup>. In the present study, we found that Maras powder use significantly decreased FMD.

Platelets play a pivotal role in atherothrombosis, the major cause of most unstable coronary syndromes<sup>(22)</sup>. Mean platelet volume, the most commonly used measures of platelet size, is a potential marker of platelet reactivity<sup>(23)</sup>. Large platelets are metabolically and enzymatically more active<sup>(23)</sup>. Smoking increased mean platelet volume<sup>(24)</sup>. In our study, mean platelet volume and platelet counts were higher in subjects who used Maras powder and cigarette smokers group than in nontobacco users subjects.

Cigarette smoking is associated with changes in blood lipids, resulting in atherogenic risk profile—primarily low HDL cholesterol<sup>(25)</sup>. Nicotine increases lipolysis and increases free fatty acid concentrations<sup>(26)</sup>. Increased fatty acid turnover is associated with overproduction of very low-density lipoprotein-total triglycerides, increased LDL-cholesterol and lowered HDL cholesterol<sup>(26)</sup>. Cross-sectional studies have shown that smokeless tobacco use seems to have an adverse effect on lipid profiles. In a study of 2840 adult men smokeless tobacco users had 2,5 times the adjusted risk of hypercholesterolemia compared with normal<sup>(27)</sup>. In another study, involving 90 percents from India, smokeless tobacco users had lower HDL cholesterol and higher triglyceride levels than control groups<sup>(28)</sup>.

Our study, in accordance with both other two studies, demonstrated that total cholesterol, LDL cholesterol and triglyceride levels were significantly higher in Maras powder group compared to control group, while HDL cholesterol levels were significantly lower.

Some studies have shown that cigarette smoking is associated with an acute and marked increase in blood pressure and heart rate<sup>(29)</sup>. Studies evaluating the effects of smokeless tobacco on blood pressure showed different results. A number of studies, supported that smokeless tobacco had a blood pressure increasing effect<sup>(30,31)</sup> whereas rest of them showed that smoking has no effect on blood pressure<sup>(32,33)</sup>. In our study, blood pressure was higher in both Maras powder and cigarette smokers group

than in nontobacco users group

Numerous studies have demonstrated an association between elevated levels of homocysteine, oxidative stress, and endothelial injury<sup>(34)</sup>. Several mechanisms have been postulated to be responsible for the negative effects of homocysteine on the endothelium. Furthermore, elevated levels of homocysteine have been shown to cause lipid peroxidation and impair vasomotor regulation, both of which transform the inner vascular wall into a prothrombotic surface, in turn resulting in atherothrombogenesis<sup>(35)</sup>. We found that, level of homocysteine was higher in subjects who used Maras powder and cigarette smokers group than in nontobacco users subjects.

Some epidemiological studies suggest that hsCRP is an important risk factor for atherosclerosis, and coronary artery disease<sup>(36,37)</sup>. Possible mechanisms of endothelial dysfunction with increased levels of hsCRP have been previously described: hsCRP is found in atherosclerotic plaque<sup>(38)</sup>, it has a direct role in the secretion of inflammatory mediators from vascular endothelium<sup>(39)</sup>, it promotes the expression of adhesion molecules<sup>(40)</sup> and opsonizes low-density lipoproteins for uptake by macrophages in atherosclerotic plaque<sup>(41)</sup>.

In our study, serum levels of hsCRP were found significantly higher in Maras powder and cigarette smokers group compared to the nontobacco users group whereas FMD measurements were significantly lower.

Several groups of individuals at high risk for atherosclerotic events, such as those with coronary artery disease, elevated LDL, hypertension, and diabetes mellitus, have been found to have endothelial dysfunction as assessed by measurement of brachial artery FMD<sup>(42)</sup>. In addition, those who smoke cigarettes and cigars have poor FMD<sup>(43,44)</sup>. Previous studies found that brachial artery FMD in cigarette smokers was approximately 4% over baseline vessel diameter, whereas the FMD of apparently healthy nonsmokers was about 11% over baseline<sup>(43)</sup>. By excluding subjects with known endothelial dysfunction, except for cigarette smokers, we attempted to reduce the effects of confounding variables on our study results.

We observed a mean brachial artery FMD of 6,5% in our group of smokers, which was similar to the FMD reported in other studies of cigarette smokers (43). The FMD of smokeless tobacco users in our study was nearly identical (5.7%) to that of smokers in our study and previous studies<sup>(43)</sup>. This

suggests that smokeless tobacco use and cigarette smoking cause a similar degree of endothelial dysfunction. Our study was not designed to determine a mechanism for impaired FMD. However, the impaired FMD observed in our smokeless tobacco users may be related to the effects of nicotine. We did not study urinary cotinine and blood nicotine levels in our study

In conclusion our results are consistent with findings in the literature in that we observed lower FMD values in smokeless tobacco users than in healthy controls, a result indicative of ED. Therefore smokeless tobacco should be accepted as harmful as cigarette smoking for cardiovascular system. We also established a negative correlation between FMD, serum hsCRP levels and homocysteine levels. As ED predicts cardiovascular morbidity, use of smokeless tobacco and cigarette smoking should be discouraged.

Our study has several limitations. First, a small number of subjects were included to study. Smaller sample size might have compromised the power of some of the analyses; results of multiple regression analyses should be interpreted with caution. Second, our findings could not be extrapolated to all tobacco users because we excluded subjects with usage of antihypertensives or other drugs (lipid lowering agents, antiagregan), diabetes, obesity, congestive heart failure, chronic obstructive lung disease, malignancies, secondary hypertension, renal failure, ischemic heart disease, peripheral vascular disease, gastrointestinal disease. Finally, patients were included from one region in Turkey, and our results may not be generalizable

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