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Research Paper

# Effects of Smokeless Tobacco "Maras Powder" Use on Nitric Oxide and Cardiovascular Risk Parameters

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## Abstract

**Background:** Smokeless tobacco use is common in various parts of the world. In Turkey a type of smokeless tobacco called "Maras powder" is widely used in southeastern region. Smoking is known to have an adverse effect on nitric oxide and cardiovascular risk factors. The aim of this study was to evaluate whether there is difference between the effects of Maras powder and cigarette smoking on the cardiovascular risk factors and nitric oxide levels.

**Methods:** In the study, participants were 48 Maras powder users, 50 cigarette smokers and 45 nontobacco user subjects. Blood samples were collected and hematological parameters and lipid parameters were measured. Plasma Nitric oxide level was also detected by using the Griess method.

**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 ($p<0.001$). Plasma Nitric oxide levels were found significantly lower in Maras powder and cigarette smokers group compared to the nontobacco user group (4.9±0.9 μmol/l, 4.8±1 μmol/l, 9.4±3.4 μmol/l, respectively, $p<0.001$) whereas there was no significant difference between the Maras powder and cigarette smokers group. In multivariate logistic regression model, cigarette smoking (Odds ratio=17.832, $p<0.001$), Maras powder usage (Odds ratio=12.311, $p=0.002$) and mean platelet volume (Odds ratio=1.425, $p=0.030$) remained independently associated with lower Nitric oxide levels.

**Conclusion:** We conclude that Maras powder has similar adverse effects on nitric oxide level and cardiovascular risk parameters and thereby it appears to be harmful as cigarette smoking.

Key words: Maras powder, smokeless tobacco, nitric oxide, cigarette.

## INTRODUCTION

There are various forms of tobacco use, which can be classified such as smoking or smokeless tobacco. Cigars, pipes and narghiles could be listed among the noncigarette preparats that produce smoke. Smokeless tobacco is a different form of tobacco use. Smokeless tobacco can be found in preparations for chewing or for being absorbed by nasal and oral mucosae (1).

Smokeless tobacco use is common in various parts of the world. Smokeless tobacco products can

range from ground tobacco mixed with spices and sugars (Chimo in Venezuela) to sodium bicarbonate (Toombak in Sudan) to areca and betel nuts (Mawa or Gutkha in India). The popularity and prevalence of specific smokeless tobacco products varies among World Health Organization (WHO) regions: gutkha in the Eastern Mediterranean Region; betel quid, gutkha, and creamy snuff in South-East Asia Region; tobacco mixed with betel nut in the Western Pacific Region; and snuff in the African Region. With the exception of Sweden and Norway, the sale and distribution of smokeless tobacco products such as moist snuff or snus is banned in most of the European Union (2).

In Turkey a type of smokeless tobacco called Maras powder is widely used in southeastern region especially in Kahramanmaras and Gaziantep cities by many addicts. Maras powder is used orally instead of cigarette smoking. This powder is known to be composed of ash of wood (especially oak, sometimes walnut) and dried leaves of plant named Nicotina Rustica Linn. First of all, sun-dried leaves of this plant are powdered and mixed with the ash in 1/2 or 1/3 proportions (tobacco and oak, respectively). Then, water is sprinkled onto this mixture for humidification. A small amount of this mixture (approximately 1gr) is applied between the lower labial mucosa and gingiva for 5-10 min and then it is spat out. This procedure is repeated many times during the day; even some people sleep with this powder (3). There were a number of studies about Maras powder and its negative effects. Maras powder negative effects on the humoral immune system (4), it increases the oxidative stress (5), and it has also genotoxic effects (6).

A number of studies and meta-analysis have shown that smokeless tobacco had an increased risk for cardiovascular diseases (7,8,9,10,11). Tobacco using is associated with the development of severe atherosclerosis possibly via mechanisms involving increased oxidative stress and nitric oxide (NO) inactivation in the vascular endothelium (12-13). NO is an endothelium-derived relaxing factor synthesized in arterial endothelium from the amino acid L-arginine by the enzyme NO synthase, which is expressed constitutively in endothelial cells (14). Endothelium-derived NO is potent endogenous vasodilator that contributes to resting arterial tone and affects both platelet function and smooth muscle cell proliferation (15). Reduction in basal NO release may cause a predisposition to hypertension, thrombosis, vasospasm, and atherosclerosis (16). In vivo, cigarette smoking and nicotine infusion impair the endothelium-dependent relaxation mediated by NO in human arteries and veins (17,18). Endothelial dysfunction is hallmark of cardiovascular disease, and NO plays critical role in determining endothelial function. But, in Turkey, there is general believe that Maras powder, which is consumed orally, is less harmful than cigarette smoking. The aim of this study was to evaluate whether there is difference between the effects of Maras powder and cigarette smoking on the cardiovascular risk factors and nitric oxide levels.

## METHODS

### Study populations

A total of 250 consecutives patients who referred to various outpatient departments (Cardiology Clinic, Public Health Clinic, Internal Medicine Clinic, Pulmonary Disease Clinic, Check-up Clinic) of Kahramanmaraş Sutcu Imam University, Faculty of Medicine within a period of 12 months using Maras powder or cigarette were included in the study. 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. Number of years for Maras powder using and cigarette smoking were recorded. Exclusion criteria were the 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, systemic illness and recent history of infection (within the last one month). Finally, 143 patients (48 Maras powder users, 50 cigarette smokers and 45 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 in the morning (between 08:00 and 10:00 AM) from peripheral veins after a 12 hours fasting period and collected in ice-cold vacuum glass tubes containing citric acid. All subjects rested at least 5-10 minutes *i*n supine position before blood sampling.

Blood samples for NO determination were centrifuged immediately at 3000g for 15 min and the serum samples was collected and frozen at -20°C until

assayed. Levels of nitrite and nitrate were measured using the Griess reaction. Briefly, for measuring total nitrite, serum samples were incubated with cadmium for 2 h, which converts all nitrate to nitrite. Samples were deproteinized, and then 100μL of Griess reagent (sulfanilamide and *N*-1-naphthylethylendiamine dihydrochloride) was added. After color development at room temperature, absorbance values were measured at a wavelength of 540 nm. Each sample was assayed in duplicate. Nitrite in the serum was estimated by a standard curve obtained from enzymatic conversion of potassium nitrate to nitrite. Results are reported as NO as micromoles per liter (19).

### Statistical analyses

Continuous variables were expressed as mean ± SD or median in the presence of abnormal distribution, and categorical variables as percentages. The NO values of the three groups were compared with one-way ANOVA. Patients were categorized into two as lower NO (≤5 μmol/l) or higher (>5 μmol/l) NO according to median value. Comparisons between groups of patients were made by use of a $\chi 2$ test for categorical variables, an independent samples t test for normally distributed continuous variables, and a Mann-Whitney U test when the distribution was skewed. Correlations were evaluated either via Pearson or Spearman's correlation tests. We used univariate regression analysis to quantify the association of variables with lower NO levels. Variables found to be statistically significant in univariate analysis were used in a multivariate logistic regression model with the forward stepwise method in order to determine the independent prognostic factors of lower NO level. A significance level of 0.05 was used for all the statistical analyses. Data were analyzed with SPSS 15.0 for Windows (SPSS Inc, Chicago, Illinois, USA).

## RESULTS

Totally 143 patients were included in the study. Maras powder group was consisted of 30 men (62.5%) and 18 women (32.5%) (mean age: 42.1±8.7 years), cigarette smokers group was consisted of 33 men (66%) and 17 women (34%) (mean age: 39.1±10.4 years), and the nontobacco users group was consisted of 20 men (44.4%) and 25 women (55.6%) (mean age: 39.4±10.6 years). There was no difference between groups according to age, gender, and BMI. Maras powder and cigarette smoking durations were similar. Plasma NO levels were found significantly lower in Maras powder (4.9±0.9 μmol/l) and cigarette smokers (4.8±1 μmol/l) group compared to the nontobacco users group (9.4±3.4 μmol/l) ($p<0.001$, $p<0.001$, respectively) whereas there was no significant difference between the Maras powder and cigarette smokers group ($p=0.300$). The baseline characteristics and NO levels of study population were shown in Table 1.

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 hemoglobin, hematocrit, 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 1).

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 lowers in Maras powder and cigarette smokers group than in the nontobacco users group (Table 1).

Pearson's correlation analysis was performed. There was a significant negative correlation between NO and age ($r=-0.205$, $p=0.014$), BMI ($r=-0.255$, $p=0.002$), systolic BP ($r=-0.305$, $p<0.001$), diastolic BP ($r=-0.309$, $p<0.001$), plasma total cholesterol ($r=-0.331$, $p<0.001$), LDL-cholesterol ($r=-0.370$, $p<0.001$), triglyceride ($r=-0.392$, $p<0.001$) levels in all patients. In addition, there was a significant positive correlation between NO and HDL cholesterol ($r=0.506$, $p<0.001$).

Results of the univariate and multivariate logistic regression analyses for predicting lower NO levels are listed in Table 2. Cigarette smoking, Maras Powder usage, mean platelet volume, male gender, systolic blood pressure, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, hemoglobin, hematocrit, and platelet counts were found to have prognostic significance in univariate analysis. In multivariate logistic regression model, Cigarette smoking (Odds ratio=17.832, 95% CI=3.562-89.272, $p<0.001$), Maras Powder usage (Odds ratio=12.311, 95% CI=2.471-61.335, $p=0.002$), and mean platelet volume (Odds ratio=1.425, 95% CI=1.036-1.961, $p=0.030$) remained independently associated with lower NO levels.

**Table 1.** Baseline of characteristic of the study population.

| | Maras Powder (n=48) | Cigarette Smokers (n=50) | Nontobacco users (n=45) | p |
|---|---|---|---|---|
| Age (year) | 42.1±8.7 | 39.1±10.4 | 39.4±10.6 | 0.258 |
| Men/women | 30/18 | 33/17 | 20/25 | 0.078 |
| Body Mass Index (kg/m²) | 25.6±2 | 25.2±2 | 24.7±1.9 | 0.071 |
| Tobacco use duration (year) | 10.3±5.4 | 12.1±7 | - | 0.193 |
| Nitric oxide (µmol/l) | 4.9±0.9(5) | 4.8±1(4.6) | 9.4±3.4(8.9) | <0.001 |
| Systolic BP (mmHg) | 132±9 | 130±10 | 121±8 | <0.001 |
| Diastolic BP (mmHg) | 82±9 | 82±10 | 74±6 | <0.001 |
| Glucose (mg/dl) | 88±9 | 88±8 | 85±8 | 0.136 |
| Total cholesterol (mg/dl) | 203±34 | 204±39 | 179±31 | 0.001 |
| Triglycerides (mg/dl) | 174±59 | 169±66 | 118±50 | <0.001 |
| HDL cholesterol (mg/dl) | 38±4 | 38±6 | 45±7 | <0.001 |
| LDL cholesterol (mg/dl) | 131±29 | 128±35 | 107±28 | <0.001 |
| Hemoglobin(g/dl) | 14±1 | 14±1.1 | 14±1 | 0.015 |
| Hematocrit (%) | 43±4 | 44±3 | 42±4 | 0.004 |
| Platelets counts (x10⁹l) | 327±45(332) | 328±48(350) | 254±43(252) | <0.001 |
| Mean platelet volumes (fl) | 9.4±1.2 | 9.6±1.3 | 7.8±0.6 | <0.001 |

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

**Table 2.** Univariate and multivariate analysis for predicting lower NO levels.

| | Univariate | | | Multivariate | |
|---|---|---|---|---|---|
| Variable | OR | (95%CI) | p | OR | (95%CI) |
| Cigarette smoking | 3.865 | 1.873-7.979 | <0.001 | 17.832 | 3.562-89.272 |
| Maras powder | 1.969 | 0.970-3.996 | 0.002 | 12.311 | 2.471-61.335 |
| Mean platelet volume (fl) | 2.021 | 1.508-2.709 | 0.030 | 1.425 | 1.036-1.961 |
| Total cholesterol (mg/dl) | 1.013 | 1.003-1.023 | | | |
| Triglycerides (mg/dl) | 1.007 | 1.002-1.023 | | | |
| HDL cholesterol | 0.905 | 0.854-0.960 | | | |
| LDL cholesterol | 1.016 | 1.005-1.028 | | | |
| Systolic BP (mmHg) | 1.043 | 1.007-1.081 | | | |
| Diastolic BP (mmHg) | 1.023 | 0.986-1.061 | | | |
| Tobacco use duration (year) | 1.023 | 0.959-1.092 | | | |
| Body mass index (kg/m2) | 1.21 | 0.946-1.329 | | | |
| Mean age (years) | 1.011 | 0.978-1.046 | | | |
| Male gender | 2.242 | 1.105-4.551 | | | |
| Glucose (mg/dl) | 1.033 | 0.993-1.074 | | | |
| Hemoglobin (g/dl) | 1.443 | 1.072-1.941 | | | |
| Hematocrit (%) | 1.107 | 1.008-1.206 | | | |
| Platelets counts (x10⁹l) | 1.011 | 1.005-1.018 | | | |

Cigarette smoking, Maras Powder usage, Mean platelet volumes, Male gender, Systolic Blood Pressure, Total Cholesterol, Triglycerides, HDL Cholesterol, LDL Cholesterol, Hemoglobin, Hematocrit, and Platelets counts were entered into the multivariate logistic regression model with forward stepwise method.
CI: Confidence interval; OR: Odds ratio.

## DISCUSSION

The principal finding of this study is that plasma NO levels were found significantly lower in Maras powder group compared to the nontobacco users group whereas there was no significant difference between the Maras powder and cigarette smokers group. Another significant finding, Maras powder, cigarette smoking and mean platelet volume remained independently associated with lower NO levels.

Maras powder is a different form of the smokeless tobacco (20). It is made of a plant N. rustica L. Nicotine content of N. rustica L is higher about 6-10 fold than N.tobacum L (21). In this case, it is mostly probable that N.rustica L is preferred in the preparation of Maras powder because of its high nicotine content. It's accepted that the ash in this mixture transforms the alkaloids into the base form and provides the absorption of them from the buccal mucosa easily (22). Cok et al. found that urinary cotinine levels were three times higher in Maras powder users than in cigarette smokers (23). We did not study urinary cotinin and blood nicotine levels in our study.

Nicotine has been reported to injure endothelial cells in vitro and in animal studies (24,25). Nicotine has been found to release growth factors and to promote angiogenesis, which could contribute to atherogenesis (26). In animals with hyperlipidemia, nicotine promotes neovascularization of vascular plaque (26). 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 (27). In our study, NO levels were significantly lower in both Maras powder group and cigarette smokers group than in nontobacco users group.

Platelets play a pivotal role in atherothrombosis, the major cause of most unstable coronary syndromes (28). Mean platelet volume, the most commonly used measures of platelet size, is a potential marker of platelet reactivity (29). Large platelets are metabolically and enzymatically more active (29). Smoking increased Mean platelet volume (30). 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. In addition we found that mean platelet volume was independently associated NO levels.

In a study of 135036 male Swedish construction workers aged 35 to 54 years, the relative risk of dying of cardiovascular causes was 1.4 times higher in smokeless tobacco users than nonusers (9). Unfortunately, the long-term studies for evaluating the effects of Maras powder on cardiovascular mortality are not available in Turkey. In Turkey, there are effective prohibition measures against smoking at public places in order to protect public health. However, Maras powder is still very commonly used because its usage can not be easily detected. In addition, unfortunately, some people who quit smoking tend to use Maras powder due to its less expensive price. This is a serious threat for public health.

Cigarette smoking is associated with changes in blood lipids, resulting in atherogenic risk profile-primarily low HDL cholesterol (31). Nicotine increases lipolysis and increases free fatty acid concentrations (32). Increased fatty acid turnover is associated with overproduction of very low-density lipoprotein-total triglycerides, increased LDL-cholesterol, and lowered HDL cholesterol (32). 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 (33). In another study, involving 90 percents from India, smokeless tobacco users had lower HDL-cholesterols and higher triglyceride levels than control groups (34). 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.

Cigarette smoking has been associated with acute insulin resistance. Although the mechanism is not entirely clear, it may be related to increased levels of norepinephrine or other counter regulatory hormones, such as growth hormone or cortisol (35,36). In our study, glucose levels were higher in both cigarette smokers and Maras powder group than nontobacco user group but the difference was not statistically significant.

Some studies have shown that cigarette smoking is associated with an acute and marked increase in blood pressure and heart rate (37). 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 (38,39), whereas rest of them showed that smoking has no effect on blood pressure (40,41). In our study, blood pressure was higher in both Maras powder and cigarette smokers group than in nontobacco users group.

## Study limitations

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, is-

chemic heart disease, peripheral vascular disease, gastrointestinal disease. Finally, patients were included from one region in Turkey, and our results may not be generalizable.

## CONCLUSION

Our results suggest that Maras powder is closely associated with traditional cardiovascular risk factors and endothelial dysfunction as detected by low plasma NO levels and therefore it should be accepted as harmful as cigarette smoking for cardiovascular system. However, further large scale studies and long term follow-up are needed to detect whether Maras powder is an independent risk factor for cardiovascular events and also it should be included to fight program against tobacco use.

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## Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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