RESEARCH ARTICLE / *Araştırma Makalesi*

The Effects of Smokeless Tobacco "Maras Powder" on Homocysteine and Cardiovascular Risk

Dumansız Tütün Maraş Otu'nun Homosistein ve Kardiyovasküler Risk Parametreleri Üzerine Etkileri

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Abstract

Introduction	This study aims to investigate the relationship between smokeless tobacco (Maras powder) and cigarette consumption with homocysteine, Paraoxonase-1 (PON-1), Arylesterase (ARE), and Lipoprotein-a (Lp (a)), which are known as risk factors for cardiovascular diseases.
Materials and Methods	The individuals included in the study were divided into three groups as Maras powder users (n = 38), cigarette smokers (n = 38), and healthy volunteers who did not use either tobacco group (n = 38). Serum homocysteine, PON-1, ARE, and Lp (a) levels of all participants were examined.
Results	When the groups are compared, the highest homocysteine level was in the Maras powder group. While the difference between the control group and the Maras powder group was statistically significant, the difference between the control and smokers groups was statistically insignificant. Although PON values were lower in both the Maras powder and smokers groups than the control group, the difference was significant only in the smoking group. ARE was significantly lower, and Lp (a) was significantly higher in both tobacco smokers groups compared to the control group.
Conclusion	Serum homocysteine levels increase in both Maraş powder users and smokers. Moreover; Decreased PON-1 and ARE levels in smokers, decreased ARE levels in Maraş powder users show that Maraş powder use has side effects at least as much as cigarettes.
Keywords	Maras powder; homocysteine; Paraoxonase-1; Arylesterase; Lipoprotein-a

Öz

Amaç	*Bu çalışma, dumansız tütün (Maraş otu) ile sigara tüketiminin kardiyovasküler hastalıklar için risk faktörleri olarak bilinen homosistein, Paraoksonaz-1 (PON-1), Arylesteraz (ARE) ve Lipoprotein-a (Lp (a)) ile ilişkisini araştırmayı amaçlamaktadır.*
Yöntem ve Gereçler	*Çalışmaya alınan bireyler Maraş otu kullananlar (n=38), sigara içenler (n=38) ve her iki tütün grubunu da kullanmayan sağlıklı gönüllüler (n=38) olarak üç gruba ayrıldı. Tüm katılımcıların serum homosistein, PON-1, ARE ve Lp (a) düzeylerine bakıldı.*
Bulgular	*Gruplar karşılaştırıldığında en yüksek homosistein düzeyi Maraş otu grubundaydı. Kontrol grubu ile Maraş otu grubu arasındaki fark istatistiksel olarak anlamlıyken, kontrol grubu ile sigara içen grup arasındaki fark istatistiksel olarak anlamsızdı. Hem Maraş otu hem de sigara içen grupta PON değerleri kontrol grubuna göre daha düşük olmasına rağmen, fark sadece sigara içen grupta anlamlıydı. Kontrol grubuna göre her iki tütün içen grupta ARE anlamlı olarak daha düşüktü ve Lp (a) anlamlı olarak daha yüksekti.*
Sonuç	*Serum homosistein düzeylerinin hem Maraş otu kullananlarda hem de sigara içenlerde artmaktadır. Ayrıca; sigara içenlerde azalan PON-1 ve ARE seviyeleri, Maraş otu kullananlarda azalan ARE seviyeleri Maraş otu kullanımının en az sigara kadar yan etkileri olduğunu göstermektedir.*
Anahtar Kelimeler	*Maraş otu; homosistein; Paraoksonaz-1; Arylesteraz; Lipoprotein-a*

INTRODUCTION

Tobacco use is still one of the most important causes of mortality and morbidity worldwide. Although tobacco smoking is mainly through cigarette smoking, other smokeless tobacco use patterns are also common. Smokeless tobacco is used by many cultures worldwide, including the United States, Sweden, India, and the Middle East.[1-4] Some common smokeless tobacco (ST) products include chewing tobacco, rappee, snus, and topical tobacco paste[3]. Maras powder (MW), an important example of the ST, is widely used in Turkey's eastern Mediterranean region. Also, other countries, such as Sudan and Saudi Arabia, often use MW as a smokeless tobacco type.[5,6] The studies conducted in Turkey reported an MW use ratio of 4 - 16.8%.[7] Unlike chewing tobacco, Maras powder is used orally. The leaves of the plant named "Nicotiana rustica Linn," which is called "Wild Tobacco, are dried and mixed with the ashes of walnut, oak or vine wood with a ratio of 1/2 or 1/3. Then, this mixture is moistened by adding a sum of water. A piece (approximately 1 g) of this mixture is placed between the lower lip mucosa and the gingiva. The mixture is spit after 4-5 minutes. Since this area's capillary blood vessels are rich where tobacco is applied, nicotine quickly enters the bloodstream. People using MW repeat this process several times a day, and some individuals even sleep all night with tobacco in their mouths. The nicotine content of N. Rustica L is approximately 6-10 times higher than N. tabacum L in cigarette.[8] In this case, N.rustica L is preferred to prepare wild tobacco due to its high nicotine content. It is assumed that the ash in this mixture converts the alkaloids into their base form and allows them to be easily absorbed from the buccal mucosa.[9]

Several studies and meta-analyses have shown that smokeless tobacco has an increased risk for cardiovascular diseases.[10-14]

It is known that homocysteine has atherogenic properties and is a risk factor for cardiovascular disease (CVD).[15] The effects of Maras powder use on the cardiovascular system have been investigated by examining many parameters, and the harmful effects of Maras powder on the cardiovascular system have been revealed.[8,16,17] However, Homocysteine, one of the known risk factors for CVD, has not been studied in Maras powder users.

Clinical studies are showing that PON-1 plays an atheroprotective role 18 and that low PON-1 activity increases the risk of CHD (coronary heart disease).[19]

In this study, we compared the serum Homocysteine, PON1, ARE, Lp (a), HDL Cholesterol, LDL Cholesterol, Total Cholesterol, and Triglyceride levels and compared Maras powder users and cigarette smokers with the control group. In this way, we aimed to contribute to the elucidation of Maras powder use's etiopathogenesis as a risk factor for atherosclerosis and other cardiovascular diseases.

MATERIALS and METHODS

Thirty-eight male subjects using Maras powder, 38 male cigarette smokers, and 38 healthy males for the control group were included in the study. The study protocol was conducted as the principles of the Declaration of Helsinki and approved by the local Ethical Committee of our hospital (21.03.2018/25). The signed informed consent form was obtained from the participants who volunteered to participate in the study.

Inclusion criteria for the study:

1. The Maras powder group: Participants using 1-2 grams of Maras powder each time, at least 3-6 times a day, were included.
2. The cigarette smoking group: Participants smoking at least one pack a day for at least one year were included.
3. The Control Group: Participants who are not on regular cigarettes or Maras powder in addition to not being a passive smoker were included.

Exclusion criteria: Patients with any type of acute or

chronic diseases, patients with medication use daily, and patients who were using both cigarettes and Maras powder at the same time were excluded. The participants' blood samples were taken after the 12-hour smoking and Maras powder-free period between 08:00 and 10:00 a.m. following the 12-hour fasting. The venous blood sampling was placed in gel blood tubes and centrifuged at 4000 g for 5 minutes. Serum samples obtained were portioned into appropriate containers and stored at -80°C until analysis. Homocysteine, Vitamin B12, and Folate levels were measured with the Roche Cobas E602 device using the electrochemiluminescence method, and the lipids were measured on the Roche Cobas C702 device by the photometric method. Paraoxonase and Arylesterase were measured by using a commercial kit on an automatic analyzer with the colorimetric principle, and Lipoprotein-a was measured with ELISA (Enzyme-Linked Immunosorbent Assay) (Rel Assay Diagnostic kit, Mega Tıp, Gaziantep, Turkey) commercial kits.

In the study, the number of samples for three different groups was determined with a: 0.05 significance level, B: 0.20, and 0.80 test power analysis. Accordingly, a total of 114 individuals, 38 individuals in each group with a 0.80 test power, were included in the study.

The compatibility of the variables to the normal distribution was examined with the Shapiro-Wilk test in the evaluation of the data. Mann-Whitney U test was used to compare two groups for the variables that did not show normal distribution. Comparisons between the three groups were made with the Kruskal Wallis H test. The statistical significance level was accepted as $p<0.05$. Statistical parameters were expressed as Median (min-max). Data were evaluated using IBM SPSS Statistics for Windows (IBM SPSS for Windows version 22, IBM Corporation, Armonk, New York, United States).

RESULTS

Demographic data are shown in Table 1. When the groups were compared, the highest homocysteine level was in the Maras powder group.

Table 1. Demographic Data

	Maras powder Median (min-max)	**Smoking Median** (min-max)	**Control Median** (min-max)	**p**
Age	38.5 (20.0-70.0)	31.0 (22.0-65.0)	30.5 (21.0-50.0)	0.098
Height	172 (162-185)	175 (159-190)	177 (163-190)	0.129
Weight	82 (55-117)	79 (62-128)	85 (55-110)	0.099
BMI	26.4 (18.9-40.0)	26.2 (19.9-39.5)	26.4 (16.8-40.0)	0.397

Kruskal Wallis H test; α:0.05 BMI: Body Mass Index

While the difference between the control group and the Maras powder group was statistically significant, the difference between the control and the smoker groups was statistically insignificant. Although PON values were lower in both the Maras powder and smokers groups than the control, the difference was significant only in the smoking group. ARE was significantly lower, and Lp (a) was significantly higher in both tobacco and Maras powder groups than the control group. (Table 2, Figure 1)

In the correlation analysis, there was a significant positive correlation between the duration of Maras powder use and cigarette smoking and homocysteine levels ($p = 0.02$, $r = 0.491$ and $p = 0.00$, $r = 0.614$, respectively) (Figure 2). There was no significant correlation between other parameters.

Table 2: Comparison of the laboratory parameters of the groups

	Maras powder	Smoking	Control	p
	Median (min-max)	Median (min-max)	Median (min-max)	
Duration (years)	11.5(2-35)	14.0(3-40)	.	0.620
Amount (pack/day)	1.0(0.5-2.0)	1.0(0.5-5.0)		0.492
Homocysteine (umol/L)	15.65(9.80-27.40) c	14.40(9.30-49.20)	13.30(10.00-19.20)a	0,026*
PON (U/L)	110.92(51.85-162.92)	91.03(5.86-354.15)c	121.61(67.35-264.58)b	0.043*
ARE (U/L)	126.67(83.30-148.97)c	123.42(44.61-211.48)c	134.89(107.78-176.74)a,b	0.011*
Lp a (mg/dL)	12.47(1.57-24.39)c	13.15(2.90-24.11)c	6.67(1.97-26.52)a,b	p<0.001*
Cholesterol (mg/dL)	167.80(114.10-239.40)	172.65(115.50-218.90)	160.75(108.00-235.20)	0.766
HDL (mg/dL)	37.50(27.20-55.30)	36.45(26.20-60.10)c	41.60(29.50-66.20)b	0.031*
LDL (mg/dL)	101.60(878.70-153.00)	95.05(57.70-145.90)	94.55(59.00-158.00)	0.549
Triglyceride (mg/dL)	167.50(15.10-439.20)	165.70(59.60-313.00)	133.65(61.80-376.00)	0.357

Mann Whitney U test: Kruskal Wallis H test; α:0.05;* Statistically significant; a significant difference with Maras powder group b significant difference with cigarette group; c significant difference with the control group
PON-1: Paraoxonase-1, ARE;Arylesterase, Lp (a);Lipoprotein-a

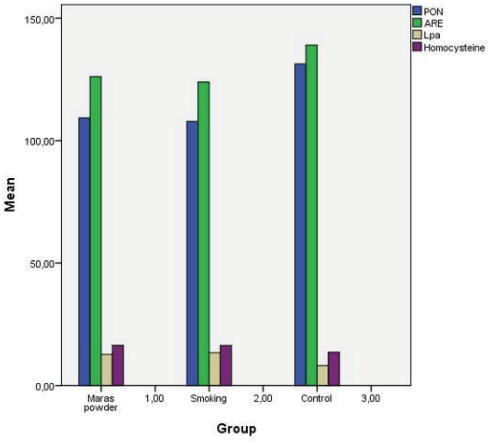

Figure 1. Comparison of the groups regarding the examined parameters

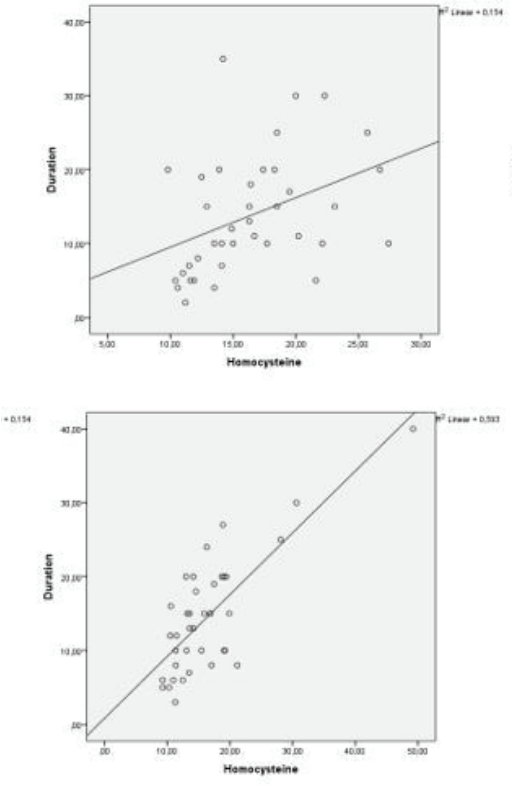

Figure 2. Relotionship between the duration of Maras powder use and cigarette smoking and homocysteine

DISCUSSION

Our study observed that homocysteine concentrations increased more in Maras powder users than cigarette smokers compared to the control group. We found that PON levels decreased with the use of Maras powder and cigarette smoking; this decrease was statistically significant only in cigarette smokers, ARE levels decreased, and Lp (a) levels increased in both Maras powder and cigarette smoking groups compared to the control. Our study is the first study to evaluate homocysteine parameters in Maras powder users.

Various studies have shown that smoking and ST use can cause cardiovascular[20], respiratory, endocrine and immune systems[9,21,22] disorders. Atherosclerosis-related diseases constitute a significant portion of cardiovascular diseases. Atherosclerosis is the most important cause of heart attacks and strokes, characterized by thickening and hardening of the arterial walls. The atherosclerotic lesion is a chronic inflammatory process. Vascular endothelium, monocytes/macrophages, smooth muscle cells, some growth factors, and cytokines are involved in this process. Although many factors play a role in atherosclerosis formation, the most important one is endothelial dysfunction. Although the exact mechanism of endothelial dysfunction is unknown, there is evidence that homocysteine, an intermediate product of methionine metabolism, exerts its effects by causing oxidative damage.[23] The increase in extracellular homocysteine is toxic to cells and tissues and can initiate a wide array of vascular complications. Vascular endothelial cells are susceptible to even a slight increase in homocysteine concentration. This sensitivity is explained by human endothelial cells' inability to express the active form of the cystathionine beta-synthase enzyme, that is, to initiate homocysteine catabolism.[24] Oxidative radicals that cause atherosclerosis are produced by homocysteine, and they can oxidize plasma LDL. Plasma oxidized-LDL (ox-LDL) increase is a well-known risk factor for endothelial dysfunction and atherosclerosis. A study conducted in a Pakistani population shows a positive relationship between ST consumption and hyperhomocysteinemia.[25] We also found that homocysteine was high in participants who use Maras powder, a type of smokeless tobacco, and this elevation suggests that it may be a risk factor for atherosclerosis and other cardiovascular diseases.

Homocysteine also triggers NADPH oxidase activity, which contributes to increased reactive oxygen species (ROS) production. Homocysteine-induced reactive oxygen species accelerate atherosclerosis development by decreasing HDL-associated PON-1 expression.[26] Paraoxonases (PON) is a family of enzymes that catalyze the same reaction using different substrates. These enzymes protect lipids from peroxidation and consequently exhibit antioxidant properties. There are three known members of the PON family; PON1, PON2, PON3. PON1 is a multifunctional enzyme with PON, diazoxonase, ARE activities. It is synthesized in the liver and reduces ROS in human endothelial cells, vascular smooth muscle cells, and fibroblasts.[27] Another PON feature is its ability to detoxify homocysteine-thiolactone, a toxic, reactive intermediate product for cells and proteins. Studies have revealed the proatherogenic and neurodegenerative effects of homocysteine with low serum PON1 activity.[28,29] Human serum PON1 level and activity are affected by diet, smoking and acute-phase proteins. Various studies have reported that PON1 levels decreased in smokers compared to non-smokers.[30,31] Again, in another study, PON1 and ARE enzyme levels were found to be low in both cigarette smokers and Maras powder users.[32] Supporting this study, we found low PON-1 levels in smokers and ARE levels in both Maras powder users and smokers in our study.

Lp (a) is a lipoprotein that contains an Apo (a) molecule disulfide-linked to Apo B100 as apolipoprotein; apart from Apo (a), its structure is similar to low-density lipoprotein (LDL). Lp (a) is synthesized in the liver. It is associated with the serum transport of cholesterol and its storage in tissues. 90% of the serum Lp (a) level is independent of the serum LDL level, and the constant plasma concentration

is under genetic control regulated by the ApoLp a locus.[33] The effects of gender and age on Lp (a) levels are minimal. Lp (a) shows homology with plasminogen; this homology causes interaction with the fibrinolytic cascade, which explains the lipoprotein's atherogenic mechanism. However, as Lp (a) is more susceptible to oxidation than LDL, the direct accumulation of Lp (a) on the artery wall is another possible mechanism.[34] Numerous studies have associated Lp (a) level with coronary artery disease.[35,36] In a study investigating the relationship between CVD risk and Lp (a) level in smokers, they found increased homocysteine and Lp (a) in smokers depending on the dose and duration of smoking. They argued that high Lp (a) and hyperhomocysteinemia, and smoking were the mechanisms that promote atherosclerosis.[37] In a study comparing Maras powder users and smokers with the control group, the highest Lp (a) value was found in the Maras powder group, and Lp (a) was interpreted as a valuable biomarker for atherosclerosis and CVD.[32] In accordance with this finding, in our study, we found Lp (a) levels higher in both the Maras powder users and the smokers' groups than the control group, but there was no difference in Lp (a) levels between the Maras powder users and smokers. Although there was an increase in plasma lipid profile in Maras powder users, the difference was statistically insignificant.

As a result, we found that serum homocysteine levels, which are considered a predisposing factor for CVD, increased in both Maras powder users and smokers in this study. This finding may be due to the breakdown of intracellular antioxidant systems or excessive free radical production. Decreased levels of PON-1 and ARE in smokers, decreased ARE levels in Maras powder users can be explained by the fact that reduce HDL-associated PON-1 expression of homocysteine-induced reactive oxygen species. Both the increase in homocysteine and Lp (a) levels and the decrease in PON1 and ARE enzyme activities may play a role in developing tobacco-related disorders such as atherosclerosis, CVD, and cancer. Our study also shows that, contrary to the belief that smokeless tobacco is less harmful than smoking, the use of Maras powder has at least as many adverse effects as smoking. Larger-scale experimental and clinical studies are needed since the use of Maras powder, and other smokeless tobacco is a potential public health hazard.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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