ORIGINAL ARTICLE

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Correlation Between the Carotid Artery Intima Media Thickness and Gamma Glutamyl Transferase Level in Maras Powder Users

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Abstract

Objective: In this study, we evaluated carotid artery intima-media thickness (CIMT), an early marker of atherosclerosis in smokers and Maras powder users, and serum gamma glutamyl transferase (GGT) levels, which is a marker of oxidative damage.

Methods: The study included 27 men who do not smoke but use maras powder, 32 men who smoke but do not use maras powder and 30 men who neither smoke nor use Maras powder as the control group. Serum levels of GGT and lipid profiles were measured and the results were compared between the groups. CIMT was measured by Doppler ultrasonography.

Results: Mean CIMT was found as 0.79±0.09 mm, 0.74±0.08 mm and 0.55±0.08 mm in Maras powder, smoking and control groups; respectively. The difference among the groups was statistically significant ($p<0.001$). Plasma levels of total cholesterol, low-density lipoprotein-cholesterol and triglyceride were significantly higher in the Maras powder and smoking groups than in the control group ($p<0.001$). Plasma levels of high-density lipoprotein-cholesterol levels were significantly lower in the Maras powder and smoking groups than in the control group ($p<0.001$). Serum GGT levels were found significantly higher in the Maras powder and smoking groups compared to the control group ($p<0.001$).

Conclusion: This study reveals that Maras powder is as effective as cigarette smoking on increasing of oxidative stress, which plays a role in the pathogenesis of many diseases. Our results suggest that CIMT is an important marker of cardiovascular complications in Maras powder users and smokers as a non-invasive method.

Keywords: Maras powder, carotid artery intima-media thickness, gamma glutamyl transferase

INTRODUCTION

Smoking is the most common type of tobacco use. However, a kind of tobacco use form, known as smokeless tobacco, presents some geographical differences in terms of usage and prevalence (1). Maras powder is a type of tobacco powder obtained from tobacco leaves, used in our region by applying through the oral mucosa. There is a common belief among native people that Maras powder is less harmful than smoking and even it is used mainly to help smoking cessation. However, as is shown in many studies, Maras powder is at least as harmful as smoking on health (2-4).

Studies have shown that many chemicals in most smokeless tobacco products and cigarets directly or indirectly lead to the formation of reactive oxygen species (ROS) (5). Normally, ROS levels are balanced through neutralization by the antioxidant defense systems of the body. When this balance is impaired in favor of ROSs, destructive reactions occur on the molecules such as proteins, lipids and nucleic acid. This condition is known as "oxidative stress" and ultimately leads to tissue damage (6). The change begins with increased carotid artery intima-media thickness (CIMT), increase because of oxidative stress in smokers and Maras powder users, causing atherosclerosis and narrowing

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and even occlusion of the lumen in the future (7). Recent studies have shown that CIMT can be used as a predictor of cardiovascular events. Moreover, the relationship between CIMT and major cardiovascular events has been proven (8).

Gamma glutamyl transferase (GGT) is an enzyme, which exists in serum and on the exterior surface of the cell membrane and catalyzes the transfer of gamma glutamyl residues of amino acids or small peptides (9). The effects of GGT on oxidative stress and glutathione metabolism is the most important mechanism that indicates the relationship between serum GGT levels and cardiovascular diseases (10).

In this study, we evaluated CIMT, an early predictor of atherosclerosis, and serum GGT levels, a marker of oxidative stress, in people who use Maras powder, which is a kind of smokeless tobacco product that contains chemicals similar to those found in cigarette. Additionally, we compared these parameters between the Maras powder and smoking groups. Several studies are available to show increased CIMT in Maras powder users, but our study is the first to examine both CIMT and serum GGT levels in the same patient group cooperatively.

METHODS

This study included 32 men as the smoking group, 27 men as the Maras powder user group and 30 healthy men as the control group. Because the use of Maras powder is common among men due to socio-cultural structure, groups were created with male subjects. The study was approved by the Local Ethics Committee of Kahramanmaras Sutcu Imam University Faculty of Medicine (decision no: 02, date: 21.03.2016). The participants were informed about the study and gave consent for carotid Doppler ultrasound (USG) screening and blood sampling.

The study groups were created from participants who have no any pulmonary symptoms, medication history and antioxidant preparations and without no underlying metabolic, endocrine, or malignant disease. Participants with underlying chronic lung disease, heart failure, hypertension (HT), malignancy, liver and kidney failure and a history of other systemic diseases were excluded from the study. Maras powder usage, duration and frequency and smoking duration and pack-year were recorded.

Ultrasound Examination

CIMTs were measured in all participants using an 8 MHz linear vascular probe in Doppler device (Mindray DC7) at Pazarcik State Hospital, Clinic of Radiology. All measurements were made by a single radiologist in the same device order to avoid inter-observer variations. USG evaluations were made after resting in the supine position for 15 min. For measurement of the carotid artery, the head was turned about 10º toward the opposite direction. USG transducer was diverted to the parallel echo lines of the intima and media walls after positioning onto the carotid arteries long axis at an angle of 90º. The distance between the lumen-intima interface and the media-adventitia interface was measured.

Laboratory Testing

5 mL venous blood samples were collected from the vessels on the forearm of each participant, placed into the biochemistry tubes and GGT levels were determined by Roche Hitachi device.

Statistical Analysis

The data were analyzed using the SPSS software. Descriptive statistics are expressed as mean and standard deviation. One-Way ANOVA test was used to determine differences between groups, followed by post-hoc Tukey HSD and Tamhane tests to detect the differences in the group with difference. $p<0.05$ values were considered statistically significant.

RESULTS

The Maras powder group included 27 (mean age: 51.1±10.4 years), smoking group 32 (mean age: 48.8±9.3 years) and control group 30 men (mean age: 47.1±11 years). There was no difference in age and body mass index (BMI) between the groups. Durations of the use of Maras powder and smoking were similar (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 users and smokers) and control group. Plasma levels of total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride were significantly higher in the Maras powder group than in the control group ($p<0.05$), whereas there was no significant difference between the Maras powder user and smoking groups. Plasma levels of high-density lipoprotein (HDL)-cholesterol were significantly lower in the Maras powder user and smoking groups compared to the control group (Table 1). Systolic arterial pressure was higher in the Maras powder user and smoking groups compared to the control group ($p<0.05$). Systolic arterial pressure values of the Maras powder group were significantly higher than those of the smoking group ($p<0.05$).

CIMT, which is suggested to be related to cardiovascular risk factors, was measured by USG and the groups were compared for CIMT values. CIMT values of the Maras powder and smoking group were significantly higher compared to that of the control group ($p<0.05$). Figure 1 shows the distribution of CIMT values according to the study groups.

Table 1. General characteristics of study population				
	Maras powder (n=27)	**Cigarette smokers (n=32)**	**Non-tobacco users (n=30)**	**p**
Age, years	51.1±10.4	48.8±9.3	47.1±11	NS
BMI, kg/m^2	27.7±4	26.3±4.1	27.3±4.9	NS
Tobacco use duration (year)	10.8±2.1	15±6.5	0	-
Systolic BP (mmHg)	135.7±12.7*	128.1±10.9	117.1±7.6	**0.001**
Diastolic BP (mmHg)	78±20.6	79.6±8.3	75±6.8	**0.03**
GGT (U/L)	52.4±21.1	42.6±20.5	20.3±11.3	**0.001**
Total cholesterol (mg/dL)	210.5±26.1	208.4±19.2	185.6±9.2	**0.001**
Triglycerides (mg/dL)	194.5±74.6	170.8±47.5	144.3±43	
HDL cholesterol (mg/dL)	36.9±6.9	38.2±3.9	48±8.3	**0.001**
LDL cholesterol (mg/dL)	139.6±25.6	133.7±27.9	106.7±12	**0.001**
CIMT (mm)	0.79±0.09	0.74±0.08	0.55±0.08	**0.001**

*p<0.05 for comparison with cigarette smokers, p=0.02. BMI: Body mass index, F: Female, M: Male, GGT: Gamma glutamyl transferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, BP: Blood pressure, CIMT: Carotid intima media thickness

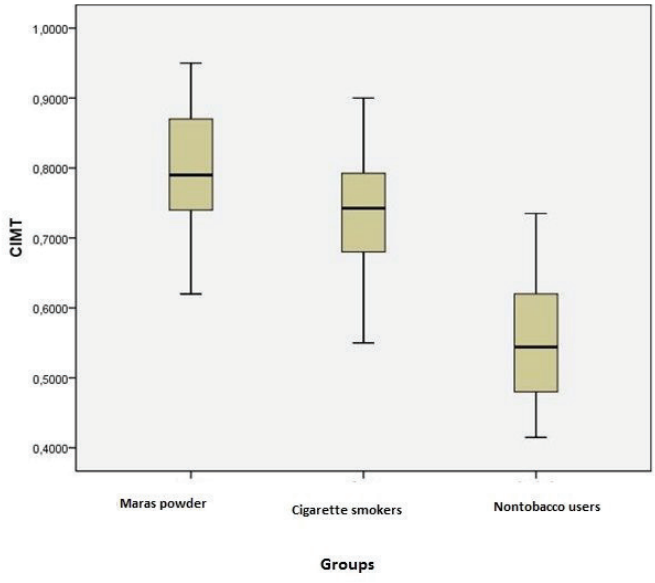

Figure 1. Distribution of CIMT values according to the study groups
CIMT: Carotid intima media thickness

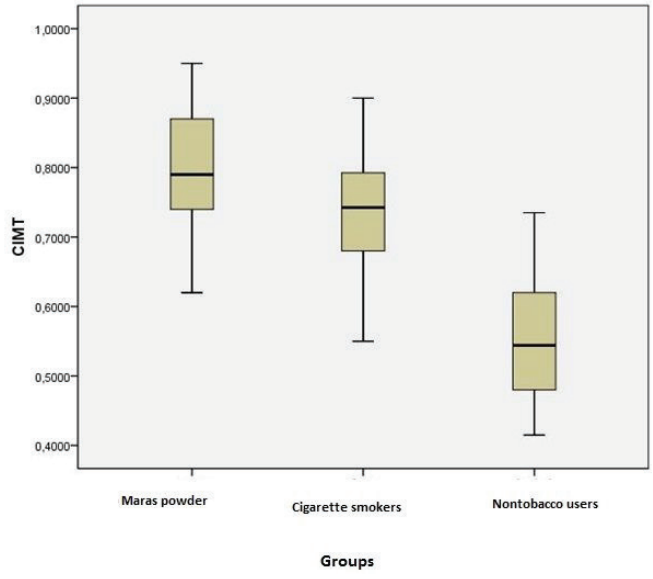

Figure 2. Distribution of serum GGT levels according to the study groups
GGT: Gamma glutamyl transferase, CIMT: Carotid intima media thickness

Additionally, serum GGT levels were evaluated to determine cardiovascular risk in the Maras powder user and smoking groups. The level of GGT was found to be significantly higher in the Maras powder user and smoking groups than in the control group (p<0.001). Figure 2 shows the distribution of serum GGT levels according to the study groups. A positive correlation was found between GGT levels and CIMT (r= 0.366, p=0.001) in Maras powder user and smoking group (Figure 3).

DISCUSSION

In this study, we evaluated the effects of Maras powder, a smokeless tobacco product, and cigarette on CIMT and serum levels of GGT. We conclude that smoking and the use of Maras powder increases CIMT.

Smoking is an important factor contributing to oxidative stress, which is caused by ROS and triggers the pathological events (11,12). It has been shown in many studies that high number of free oxygen radicals due to smoking, increase lipid peroxidation and oxidative DNA damage in the cell. Maras powder contains carcinogenic and mutagenic agents in varying amounts as in cigarette (13). Therefore, endothelial dysfunction occurs because of the formation of free radicals and subsequently developing oxidative stress due to smoking and the use of Maras powder. Endothelial dysfunction also predisposes to atherosclerosis. Studies have shown that oxidative stress plays an important role

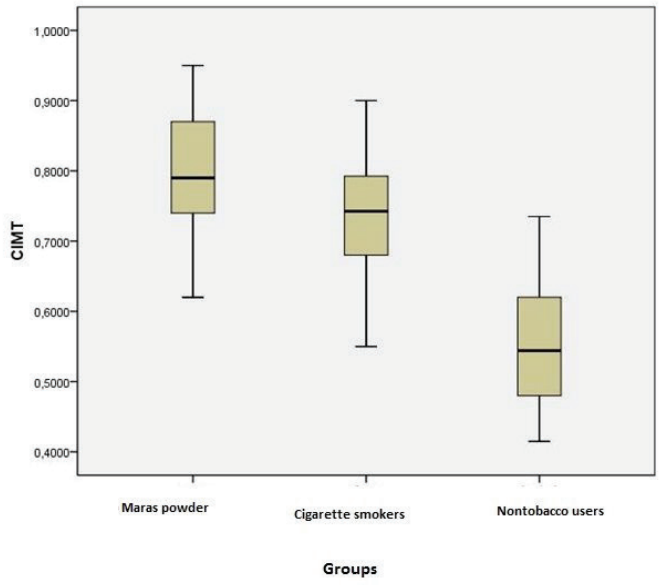

Figure 3. Correlation of GGT levels and CIMT
GGT: Gamma glutamyl transferase, CIMT: Carotid intima media thickness

in the pathogenesis of atherosclerosis. LDL in serum is converted to the oxidized LDL, an atherogenic form by oxidation, after which the accumulation of oxidized products in macrophages form foam cells and finally atheroma develops (7).

The primary role of GGT, which is a critical diagnostic test for the diagnosis of gallbladder disease, is to destroy the reduced glutathione, which is outside the cell and to provide precursor amino acids for the synthesis of intracellular glutathione. Furthermore, GGT is an important enzyme that mediates the regulation of important reductive events such as antioxidant/anti-toxic defense and cell proliferation/apoptosis balance (14,15). In recent experimental studies, it has been reported that cellular GGT might increase the formation of ROS in the presence of iron and other transition metals. In an animal study, it was been claimed that GGT activity might be a marker for oxidative stress because GGT expression is significantly increased by oxidants in rat lung epithelial cells (16). Eventually, use of increased serum GGT activity as a marker of oxidative stress in humans has been suggested (17,18).

Oxidative stress is increased due to smoking and is effective in the development of smoking-related diseases, has been shown in many studies to be also increased by Maras powder use. Köse et al. (6) found that the Maras powder is actually effective in increasing oxidative stress and that serum total antioxidant capacity levels are decreased as the duration of maras powder use increases. In parallel to this work, Samal et al. (19) showed that in their study including 60 healthy male chewing tobacco, increased erythrocyte malondialdehyde levels depending on the duration of use and decreased erythrocyte superoxide dismutase and glutathione reductase levels that resulted in increased oxidative stress. Yildiz et al. (20) found that glutathione and malondialdehyde levels are significantly decreased in smokeless tobacco product users. Similarly, we found in our study that the serum GGT levels in Maras powder users and smokers were significantly increased compared in the control group.

The effects of GGT on oxidative stress and GSH metabolism are the most important mechanisms demonstrating the relationship between serum GGT and cardiovascular diseases. In some studies, GGT activity has been detected in the carotid and coronary artery atheromas (21). It was claimed that GGT present in atheromas might contribute to plaque formation and rupture by catalyzing the oxidation of the lipoproteins (22). CARDIA study has revealed a strong and positive correlation between serum GGT levels and traditional markers of oxidative stress such as the levels of C-reactive protein, uric acid, and fibrinogen (23). Similarly, according to Emdin et al. (24) GGT level has an independent predictive value for mortality and the incidence of non-fatal myocardial infarction in patients with a history of myocardial infarction and documented coronary artery disease. The prognostic significance of GGT levels for overall and cardiac related mortality has been shown by a large study including middle-aged men (25). Our study revealed a positive correlation between serum GGT levels and CIMT as an early and non-invasive predictor of atherosclerosis in smokers and Maras powder users.

CIMT increases in the presence of risk factors for atherosclerosis, such as age, cholesterol, diabetes mellitus, HT and smoking. Studies have shown the use of Maras powder as another reason. Sucakli et al. (26) showed increased CIMT due to the use of maras powder and found a positive correlation between CIMT and systolic/diastolic arterial pressure. Similarly, in our study, CIMT and systolic arterial pressures were higher in the smoker and Maras powder user groups compared to the control group. The increase in systolic and diastolic arterial pressure is closely associated with increased CIMT (27). It may be considered that increased CIMT by the use of Maras powder is a result of acute HT due to adrenaline release and subsequent stimulation of the sympathetic nervous system caused by the high nicotine content of Maras powder (28).

The use of Maras powder and smoking adversely affect LDL cholesterol and triglyceride levels, which have been mainly blamed in many studies for developing atherosclerosis. Similarly, in our study, plasma levels of total cholesterol, LDL cholesterol and triglyceride levels were significantly higher in the Maras powder user group compared to the control group, whereas

there was no significant difference between the Maras powder user and smoking group. Plasma levels of HDL-cholesterol were significantly lower in the Maras powder user and smoking groups compared to the control group.

Study Limitations

There are some limitations to our study. First, it was conducted with a relatively small number of subjects that was likely inadequate to reflect cardiovascular complications in Maras powder users and smokers. Additionally, we excluded patients whose BMI less than 25. We believe that further studies with larger sample sizes are needed to confirm these findings. Furthermore, cross-sectional structure of the data did not allow to establish causal links.

CONCLUSION

CIMT measurement is an easily applicable non-invasive method that enables the early detection of atherosclerotic changes in the vascular bed. Our results suggest that CIMT measurement is important in terms of being a non-invasive examination method which can be used to detect cardiovascular complications in Maras powder users and smokers. People traditionally start to use Maras powder at an early age and continue throughout life. It is generally thought to be harmless or less harmful than cigarettes, so people use Maras powder as a smoking cessation method or to reduce the harmful effects of smoking. However, we concluded with the results of the current study that Maras powder is as dangerous as smoking.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee of Kahramanmaras Sutcu Imam University Faculty of Medicine (decision no: 02, date: 21.03.2016).

Informed Consent:

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.B., Concept: F.B., N.D., Design: F.B., N.D., A.K., Data Collection or Processing: F.B., A.K., Analysis or Interpretation: F.B., N.D., Literature Search: F.B., N.D., Writing: F.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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