

Evaluation of the effects of chronic biomass fuel smoke exposure on peripheral endothelial functions: an observational study

Kronik biyokütle yakıt dumanı maruziyetinin periferik endotelial fonksiyonlar üzerindeki etkilerinin değerlendirilmesi: Bir gözlemsel çalışma

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ABSTRACT

Objective: To evaluate the effect of chronic biomass fuel (BMF) smoke exposure on peripheral endothelial functions.

Methods: Forty-seven healthy subjects who have been exposed to BMF smoke since birth (mean age 31.6±6.8 years, 21 male) were enrolled in the present cross-sectional observational study. The control group consisted of 32 healthy subjects (mean age 27.9±4.4 years, 11 male). The carotid intima media thickness (CIMT), flow associated dilatation (FAD %) and endothelium independent vasodilatation (GTN %) were assessed in all subjects. The carotid CIMT was defined as the distance between the leading edge of the lumen-intima and the media-adventitia interfaces. FAD % was defined as the percentage change in the internal diameter of the brachial artery during reactive hyperemia related to the baseline. GTN % was defined as the change in diameter in response to the application of 400 µg of glyceril trinitrate relative to the baseline scan at the end of the fourth minute. Statistical analysis was performed using Student's t-test, Chi-square test and Spearman rank order correlation analysis.

Results: The average exposure time of the subjects to biomass fuel smoke was 31.7±6.6 years. They have been exposed to dung inhalation products meanly 8.3±1.8 months in a year seasonally. The average daily exposure time was 15.7±3.3 hours. CIMT values of the two groups were not statistically different from each other (0.47±0.09 vs. 0.49±0.06 mm, p=0.138). However, a markedly reduced FAD % was determined in the study group (5.06±4.95 vs. 10.7±4.64, p<0.001). And GTN % of the BMF exposed group was significantly lower than the control group (14.41±8.47 vs. 21.85±7.87, p<0.001).

Conclusion: FAD % and GTN % are markedly reduced in the individuals who have been exposed to BMF smoke inhalation products. Therefore, chronic BMF smoke exposure may be a risk factor for the development of endothelial dysfunction. (*Anadolu Kardiyol Derg* 2011; 11: 492-7)

Key words: Biomass fuel smoke exposure, endothelial dysfunction, early atherosclerosis, air pollution, adverse effects

ÖZET

Amaç: Hayvansal biyokütle yakıtı (tezek) dumanı inhalasyon ürünlerine kronik olarak maruz kalan bireylerde endotelial fonksiyonların değerlendirilmesi.

Yöntemler: Doğumlarından itibaren tezek dumanı inhalasyon ürünleri maruziyeti olan 47 sağlıklı birey (ortalama yaş 31.6±6.8 yıl, 21 erkek) enine kesitli gözlemsel çalışmaya dahil edildi. Kontrol grubu ise 32 sağlıklı kişiden (ortalama yaş 27.9±4.4 yıl, 11 erkek) oluşturuldu. Tüm bireylerde, karotis intima media kalınlığı (CIMT), akım ile ilişkili dilatasyon (FAD %) ve endotel- bağımlı olmayan vazodilatasyon (GTN %) değerlendirildi. CIMT, lümen-intima ile media-adventisya yüzeylerinin önde gelen kenarları arasındaki mesafe olarak hesaplandı. FAD %, reaktif hiperemi sırasında brakial arter lümen çapının başlangıç düzeyine göre yüzde değişimi olarak tanımlanırken, GTN % ise, 400 µg gliseriltrinitrat uygulandıktan dört dakika sonra ölçülen brakial arter lümen çapının başlangıç düzeyine göre yüzde değişimi olarak tanımlanmıştır. İstatistiksel analiz Student t-testi, Ki-kare testi ve Spearman korelasyon katsayısı analizi ile yapıldı.

Bulgular: Her iki grubun CIMT değerleri arasında istatistiksel açıdan anlamlı farklılık yoktur (0.47±0.09'a karşın 0.49±0.06 mm, p=0.138). Ancak, kontrol grubu ile karşılaştırıldığında, çalışma grubundaki kişilerin FAD % değerleri belirgin azalmış olarak saptanmıştır (5.06±4.95'e karşın 10.7±4.64, p<0.001). Buna ilaveten, tezek maruziyeti olan kimselerin GTN % değerleri de kontrol grubuna göre düşük düzeylerde bulunmuştur (14.41±8.47'ye karşın 21.85±7.87, p<0.001).

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Sonuç: Tezek dumanı inhalasyon ürünlerine kronik olarak maruz kalan bireylerde FAD % ve GTN % değerleri belirgin olarak azalmıştır. Bundan dolayı, tezek dumanı inhalasyon ürünlerine kronik maruziyet, endotel disfonksiyonu gelişimi için bir risk faktörü olabilir. (*Anadolu Kardiyol Derg 2011; 11: 492-7*)

Anahtar kelimeler: Hayvansal biyokütle yakıtı dumanı maruziyeti, endotel disfonksiyonu, erken ateroskleroz, hava kirliliği, yan etkiler

Introduction

Biomass fuel (BMF) is plant or animal material; wood, charcoal, dung and crop residues account for more than one half of domestic energy in most developing countries and about 2.4 billion people use BMF as their main source of domestic energy for cooking, heating and lighting (1). Dried animal dung is a kind of BMF, which is used extensively at rural areas of eastern Turkey and exposure to inhalation products of BMF from birth is common. Inefficient burning of BMF in an open fire or a traditional stove generates large amounts of particulate matter as well as carbon monoxide, hydrocarbons, oxygenated and chlorinated organics and free radicals. Indoor effects of these products have been investigated and an increase in the incidence of chronic obstructive pulmonary disease, asthma, low birth weight, interstitial lung disease, lower respiratory tract infections and cataract was declared (2-8). Although there is a paucity of data on the association between cardiovascular disease and BMF, it has been shown that particulate air pollution leads to rapid and significant increases in fibrinogen, plasma viscosity, platelet activation and release of endothelins, which may indicate BMF as a considerable risk for cardiovascular health (9-11).

Endothelial dysfunction is an early event in atherosclerosis and there is a close relation between coronary artery disease (CAD) and peripheral endothelial dysfunction, which reflects the functional impairment of the endothelium before morphological changes can be detected. Carotid intima-media thickness (CIMT), flow associated dilatation (FAD %) and nitric oxide sensitivity of the smooth muscle cell of the brachial artery to nitroglycerine (GTN %) are legitimate markers of early atherosclerosis (12-24). CIMT is a surrogate marker for generalized atherosclerosis and imparts prognostic information independent of traditional cardiovascular risk factors. FAD % is a sensitive predictor of CAD, although it is unable to predict both the extent and the severity of angiographically assessed CAD (12-14). GTN % is an index of endothelium independent vasodilatation and a reduced GTN % is a valuable marker in predicting more advanced coronary atherosclerosis (12-24).

Although there are many studies about the association between BMF smoke exposure and pulmonary diseases in the adults, there are only few data concerning the effects of BMF smoke on cardiovascular atherosclerotic diseases.

In our study, we evaluated the effects of chronic BMF smoke exposure in a group of individuals living in a rural area of northeastern part of Turkey, by measuring CIMT, FAD % and GTN % as surrogate markers of early atherosclerosis.

Methods

Study design

This observational cross-sectional study was carried out in Ardahan, a small city in the northeastern part of Turkey between January and July 2008.

Study population

Forty-seven healthy subjects (26 female, 21 male), who live in a rural area in the northeastern part of Turkey and have been exposed to biomass smoke inhalation products since birth were included in the study group. The mean age was 31.6 ± 6.8 years. These subjects used BMF (dried animal dung) as their main source of domestic energy for cooking and heating with poor indoors ventilation. As the winter season is long and heavy in this region, male and female subjects had spent most of their time indoors. They were exposed to dung inhalation products meanly 8.3 ± 1.8 months in a year seasonally. The average daily exposure time was 15.7 ± 3.3 hours.

The control group consisted of 32 healthy subjects (21 female, 11 male) with no exposure to biomass inhalation products. The mean age was 27.9 ± 4.4 years.

Control group and exposed subjects were living in the same area at the time of the study; however, the control group had spent most of their lives in the big cities in the western part of Turkey, while the study group had lived in the rural area of interest since birth. There was no polluting industry within 3 km radius of the study area.

Subjects with the possibility of coronary artery disease after medical history, physical examination, electrocardiographic and echocardiographic examinations were excluded from the study (subjects having anginal symptoms, ischemic findings in the electrocardiogram or pathological findings in the echocardiography). Exclusion criteria were active and passive smoking, alcohol consumption, history of any known cardiovascular disease including coronary artery disease, hypertension, valvular heart disease, myocardial or pericardial disease, arrhythmia, and diabetes mellitus. Patients with concomitant inflammatory diseases such as infective and autoimmune disorders, neoplastic diseases, major depression, liver and kidney diseases and recent major surgical procedure, dyslipidemia and/or history of cholesterol-lowering therapy such as statins were also excluded from the study.

Local ethics committee approved the study, and written consent was obtained from each subject.

Study protocol

All the participants underwent a detailed medical history and physical investigation by the investigator doctors. Height

and weight were measured and body mass index (BMI) was calculated dividing weight in kilograms by height in meters squared (kg/m^2). Blood pressure of the participants was measured 3 times, 5 minutes apart after 10 minutes rest and average of three measurements was recorded. Electrocardiograms and transthoracic echocardiographic examinations were performed. Venous blood samples of the subjects were collected from the antecubital vein resting in the supine position after 12 hours fasting. The blood was drawn simultaneously from both groups for determination of serum total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, and triglyceride levels.

CIMT measurement

Ultrasound studies were performed (22) with a 10 MHz linear-array transducer (SDU-2200 Pro; Shimadzu, Kyoto, Japan) in a room temperature of $23\pm 2^\circ\text{C}$. The subjects were in supine position during the procedure. The common carotid artery was scanned bilaterally in longitudinal projections, and six different blinded measurements were taken about 1 cm proximal to the bifurcation. The image was focused on the posterior wall of the artery. The CIMT was defined as the distance between the leading edge of the lumen-intima and the leading edge of the media-adventitia interfaces. The intra-observation coefficient of variation for repeated measures of CIMT was 2%.

Brachial artery ultrasound measurements

1. Rest brachial artery lumen diameter: Brachial artery was scanned longitudinally. The lumen diameter was measured in millimeters as the distance between the anterior wall intima-lumen interface and the posterior wall intima-lumen interface. The lumen diameter was measured at 10 minutes of rest.

2. FAD % measurement: FAD % is the change in arterial diameter relative to the baseline scan in response to reactive hyperemia as an endothelium dependent stimulus to vasodilatation caused by an increase in shear stress. To induce increased flow, a blood pressure cuff was placed around the forearm and inflated to a pressure of about 250 mm Hg for 2 minutes. After cuff release, the arterial diameter was measured at 40, 60 and 80 seconds. Average of the 3 measurements was calculated to derive the maximum FAD (23, 24). The brachial artery was then allowed to return to normal for ten minutes, and repeat baseline images were obtained.

3. Endothelium independent vasodilatation induced by nitroglycerin (GTN %): Subsequently, a third measurement was made immediately following sublingual administration of 400 μg glyceril trinitrate spray to induce endothelium-independent vasodilatation. The diameter was measured again after four minutes. The change in arterial diameter after glyceril trinitrate administration was defined as the change in diameter in response to the application of 400 μg of glyceril trinitrate relative to the baseline scan at the end of the fourth minute (24).

Statistical analysis

All statistical studies were carried out with SPSS program version 10.0 (SPSS Inc., Chicago, IL, USA). Data are given in mean \pm standard deviation and categorical data are expressed as percentages. The Student t-test was used for comparing two independent variables and Chi-square test was used to analyze categorical data. The correlation analysis between variables was performed by using Spearman Rank Order Correlation method. A two-tailed p less than 0.05 ($p\leq 0.05$) was considered statistically significant.

Results

Baseline characteristics (Table 1)

The clinical characteristics, blood pressures and blood lipid levels of the study and control groups are summarized in Table 1. The mean age of the study group was slightly, but significantly ($p<0.01$) higher than the mean age of control group. Body mass index (BMI), systolic blood and diastolic blood pressures were similar in the both groups. Serum total cholesterol and LDL-cholesterol levels in the study group were slightly higher ($p=0.008$ and $p=0.02$, respectively) than the values observed for the control group (Table 1), while serum HDL-cholesterol and triglyceride levels were comparable and there was no statistically significant difference between the two groups for these parameters ($p>0.05$).

CIMT and endothelial function (Table 2)

Table 2 demonstrates the CIMT, brachial artery diameter at rest, brachial artery diameter after cuff release, FAD %, brachial artery diameter after sublingual glyceril trinitrate administration, and GTN % values in the study and the control groups. The mean CIMT was 0.47 ± 0.09 mm for the study group and 0.49 ± 0.06 mm for the control group and there was no statistically significant difference between the two groups ($p>0.05$).

The mean brachial artery diameter at rest and after cuff release in the study group was significantly ($p<0.001$) wider than the values observed in the control group. In the study group, FAD % mean value was $5.06\pm 4.95\%$, about half of ($p<0.001$) the value $10.80\pm 4.67\%$ observed in the control group.

The mean brachial artery diameter after sublingual GTN administration in the study group was, 4.38 ± 0.56 mm, significantly ($p<0.001$) narrower than the value 4.91 ± 0.62 mm, observed in the control group. The mean GTN % in the study group was significantly lower ($p<0.001$) than the value observed in the control group (Fig. 1).

The relations between age, BMI, CIMT, FAD % and GTN % were analyzed by Spearman's method for the study and control groups separately. In the study group, the correlation analysis revealed no significant relation between age and CIMT ($r=0.038$; $p=0.799$), age and FAD % ($r=0.198$; $p=0.181$), age and GTN % ($r=0.068$; $p=0.648$), BMI and FAD % ($r=-0.026$; $p=0.862$), BMI and GTN % ($r=0.012$; $p=0.937$), BMI and CIMT ($r=0.156$; $p=0.294$), CIMT and FAD % ($r=0.096$; $p=0.517$),

Table 1. Demographic and clinical data

Variables	Study Group (n=47)	Control Group (n=32)	p*
Age, years	31.7±6.6 (33; 16-40)	28.4±3.8 (29; 22-36)	0.012
BMI, kg/m ²	23.1±3.5 (22; 19-28)	22.5±1.6 (22; 20-26)	0.414
Smoking, n (%)	None	None	-
Smoker in family, n (%)	None	None	-
Alcohol drinking, n (%)	None	None	-
Marital status (married), n	31	18	-
School education duration, years	5-11	11-16	-
Family income, TL/month	500-1500	750-4000	-
Type of cooking fuel	Animal dung	LPG	-
Systolic blood pressure, mm Hg	119±11	114±12	0.054
Diastolic blood pressure, mm Hg	67±8	68±8	0.673
Total cholesterol, mg/dl	178±26	161±30	0.008
LDL-cholesterol, mg/dl	110±26	95±30	0.021
HDL-cholesterol, mg/dl	53±13	54±11	0.650
Triglyceride, mg/dl	92±40	104±45	0.194

Data are presented as mean±SD, median (range) and number (percentage)
*Unpaired Student's t and Chi-square tests
BMI - body mass index, HDL - high-density lipoprotein, kg/m²- kilograms/height, LDL - low-density lipoprotein, TL - Turkish lira

Table 2. Endothelial function parameters

Variables	Study Group (n=47)	Control Group (n=32)	p*
CIMT, mm	0.47±0.09	0.49±0.6	0.127
BA rest diameter, mm	4.29±0.53	3.59±0.44	<0.001
BA diameter after cuff release, mm	4.52±0.55	3.98±0.48	<0.001
BA diameter after GTN, mm	4.91±0.62	4.38±0.56	<0.001
FAD %, %	5.06±4.95	10.80±4.67	<0.001
GTN %, %	14.41±8.47	21.98±7.95	<0.001

Data are presented as mean±SD
*Unpaired Student's t
BA - brachial artery, CIMT - carotid intima-media thickness, FAD - flow associated dilatation, GTN - endothelium independent vasodilatation

or CIMT and GTN % ($r=0.077$; $p=0.605$). There was also no significant relationship between any of these variables in the control group ($p>0.05$ for all of these variables.)

There was positive and highly significant correlation between FAD % and GTN %, in both control ($r=0.762$; $p<0.001$) and study ($r=0.587$; $p<0.001$) groups.

Discussion

These results demonstrate endothelial and smooth muscle cell functions in the peripheral arterial system are altered nega-

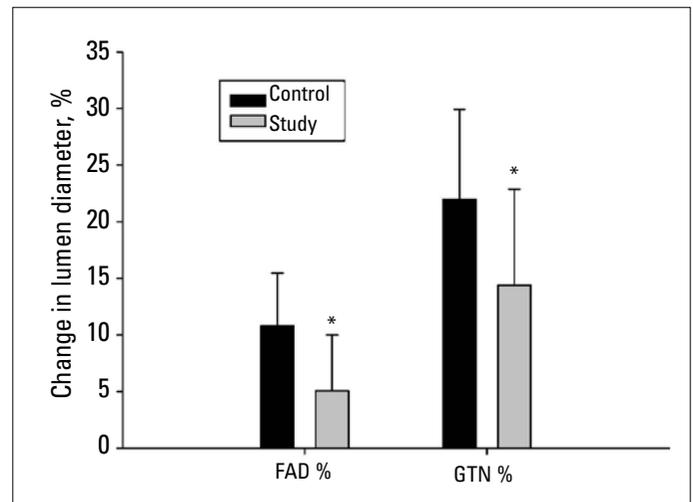


Figure 1. FAD % and GTN % values in the control and study groups

tively as a consequence of chronic BMF smoke exposure. FAD % (indicator of endothelial function) and GTN % (indicator of smooth muscle cell function) values of the subjects exposed to BMF (dung) smoke chronically for their life time, 16-40 years, were nearly half of the control group values. Reduced FAD % and GTN % values were not associated with increase in CIMT, a marker for generalized advanced atherosclerosis.

Endothelial dysfunction is an early event in atherosclerosis and there is a close relation between CAD and peripheral endothelial dysfunction. Endothelium dependent vasodilatation is mediated through endogenous vasodilators such as nitric oxide generated by endothelial cells (25). Flow mediated dilatation is a predictor of early atherosclerosis determined by the measurement of FAD % non-invasively. A reduced FAD % is determined in early stages of CAD with a sensitivity of 90%, a specificity of 37% and a negative predictive value of 43%. FAD % is a sensitive predictor of CAD (12-14), although it is unable to predict both the extent and the severity of angiographically assessed CAD (26, 27). In our present study, the study group has shown a reduced FAD % indicating reduced flow mediated vasodilatation responsiveness, which reflects endothelial dysfunction and may suggest early stages of atherosclerosis.

The exact mechanism(s) of the reduced FAD % in humans, who are chronically exposed to BMF smoke, is not known. However, it is known that burning of dried animal dung by lack of ventilation in houses results in elevations of particulate matters, carbon monoxide, hydrocarbons, oxygenated organics and free radicals (28-30). Circulating levels of oxidative stress markers [i.e., malonyldialdehyde (31), protein carbonyl (32)] increase in patients, who have been exposed to BMF smoke (31, 32). It has also been shown that exposure to BMF smoke brings out higher fibrinogen and endothelin levels and enhances platelet activity (9-11). Thus, taken together, it is reasonable to suggest that the observed impairment in the endothelial functions could result from chronic exposure to toxic particulate matters, hydrocarbons, oxygenated organics, free radicals (28-30), and carbon monoxide (33), oxidative stress (31, 32), increased endothelin

levels and plasma viscosity, hyperfibrinogenemia, and enhanced platelet activity (9-11).

Unlike FAD %, GTN % is an index of endothelium independent vasodilatation and shows the responsiveness of smooth muscle in peripheral arterial system to exogenous nitric oxide (i.e., glyceril trinitrate). It is known that advanced coronary atherosclerosis is associated with systemic atherosclerosis presenting with dysfunction of the smooth muscle cells of the peripheral arterial system (34). Thus, GTN % is not only a parameter of reduced nitric oxide sensitivity in the smooth muscle cell of the arterial wall, but a reduced GTN % is also a valuable marker in predicting more advanced coronary atherosclerosis. In the present study we showed that GTN % was reduced markedly in chronic BMF smoke exposed subjects. Observed decrease in GTN % indicates the impairment in the smooth muscle functions in peripheral arterial system, in addition to endothelial dysfunctions as suggested by the reduced FAD % (see above). This impairment in the muscle functions may be explained by direct toxic effects of BMF products on smooth muscle cells of the arteries leading to a poor vasodilatation capacity and/or presence of atherosclerosis, which is known to be associated with dysfunction of the smooth muscle cells of the peripheral arterial system (34).

CIMT is a surrogate marker for generalized atherosclerosis and imparts prognostic information independent of traditional cardiovascular risk factors (15-22). CIMT increases continuously with the extent of coronary artery disease (23). In a recent study, Davutoğlu et al. (33), reported that a group of non-smoker indoor barbecue workers who were exposed to chronic carbon monoxide had increased levels of CIMT. In the present study CIMT values were similar in the study and control subjects. This was expected since, as we noted in the methods section, we excluded the subjects with the diagnosis and possibility of coronary artery disease after medical history, physical examination, electrocardiographic and echocardiographic examinations (subjects having anginal symptoms, ischemic findings in the electrocardiogram, or pathologic findings in the echocardiography). Although the reduction in FAD % and GTN % suggests presence of some degree of CAD and atherosclerosis, CIMT data suggests that CAD and/or systemic atherosclerosis are not severe if present in our subjects.

Study limitations

Several limitations of the present study deserve consideration. First, although our results demonstrate a close relation between chronic biomass fuel exposure and peripheral endothelial dysfunction in terms of reduced FAD % and GTN %, long term prospective follow-up studies are needed with more participants to clarify our findings. Second, we did not have the possibility to determine the serum levels of biomass fuel smoke inhalation products such as carbonmonoxide, nitric oxide, hydrocarbon, and oxygenated organic levels by evaluating blood samples. Third, power analysis of the study was not performed.

Conclusion

In conclusion, the present study demonstrates that peripheral endothelial function is altered markedly as a consequence of chronic biomass fuel smoke exposure. The present study is the first to show that chronic biomass fuel smoke exposure reduces FAD % and GTN %, indicating impairment in endothelium dependent and endothelium independent vasodilatation functions, respectively. FAD % and GTN %, particularly FAD %, are also valuable indexes of early atherosclerosis. This data deserves further studies and long term follow-up for clarifying possible links between chronic biomass fuel smoke exposure and peripheral endothelial dysfunction as well as CAD and atherosclerosis.

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Conflict of interest: None declared.

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