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Original Article

Acute effect of moderate exercise on oxidative stres in smoker versus non-smokers

Sigara içen ve içmeyen bireylerde orta derecede egzersizin oksidatif stres üzerine akut etkileri

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ABSTRACT

Aim: : One of the particular sources of oxidative stress is smoking and thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) have been used as biomarkers of lipid peroxidation of oxidative damage. Therefore, we aimed to investigate the acute effect of moderate exercise on oxidative stress by determining serum MDA levels by TBARS in moderate smoker subjects versus non-smokers.

Material and Methods: Fifty healthy subjects performing moderate intensity exercise were assigned to 2 groups as moderate smokers (11-20 cigarettes/day) (Group Smoker, n=25) and non-smokers (Group Non-Smoker, n=25). Venous blood samples were collected from all participants half an hour before exercise (pre-exercise) and immediately after exercise (post-exercise) to determine MDA levels as an indicator of lipid peroxidation in the serum by TBARS/UV (ultra violet).

Results: Although no significant differences were observed in pre-exercise MDA levels between the groups, post-exercise MDA levels in smokers were significantly higher than that of non-smokers ($p<0.05$).

Conclusion: The MDA determination in serum by TBARS/UV appears to be positively correlated with smoking status in particularly female subjects. Therefore, it can be a promising helpful tool in demonstrating the oxidative stress due to moderate exercise particularly in smokers to reorganize a healthier life style.

Key words: Malondialdehyde (MDA), Smoking, Exercise, Oxidative Stress

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ÖZ

Amaç: Oksidatif stresin esas kaynaklarından biri sigara içilmesidir ve tiyobarbitürik asit reaktif türevleri (TBRAT) ve malondialdehit (MDA) oksidatif hasarın biyobelirteci olarak kullanılmıştır. Bu nedenle sigara içen ve içmeyen bireylerde TBART ile serumda MDA seviyelerini belirleyerek orta derecede egzersizin akut etkilerini araştırmayı amaçladık.

Gereç ve Yöntemler: Orta derecede egzersiz yapan ve orta derecede sigara içen (11-20 sigara/gün)(Grup Sigara İçen, n=25) ve hiç sigara içmeyen (Grup Hiç Sigara İçmeyen, n=25) 50 sağlıklı birey iki gruba ayrıldı. Tüm bireylerden egzersizden yarım saat önce ve egzersizden hemen sonra TBART/UV (ultra viole) ile serumda lipid peroksidasyonu indikatörü olarak MDA seviyelerini belirlemek için venöz kan örnekleri alındı.

Bulgular: Gruplar arasında egzersiz öncesi MDA seviyelerinde anlamlı değişiklik olmamasına rağmen sigara içenlerde egzersiz sonrası MDA seviyeleri, sigara içmeyenlerden istatistiksel olarak anlamlı şekilde yüksek bulundu ($p<0.05$).

Sonuç: TBART/UV ile serumda MDA seviyelerinin belirlenmesi, özellikle kadın bireylerde sigara içilmesiyle pozitif ilişki göstermektedir. Bu nedenle özellikle sigara içenlerde daha sağlıklı bir hayat tarzını organize etmek için orta derecede egzersize bağlı oksidatif stresin gösterilmesinde ümit vaad eden yararlı bir araç olabilir.

Anahtar Kelimeler: Malondialdehit (MDA), Sigara, Egzersiz, Oksidatif stres

Introductıon

Oxidative stress is defined as an imbalance between oxidants and antioxidants on the cellular base (1). There are various exogenous oxidative stress inducers including UV (ultra violet), radiation, inflammation, air pollution, physical exercise and smoking which can result in the formation of free radicals (2). Basically, smoking and exercise are two factors result in oxidative stress or damage (3, 4). Tobacco smoke contains gas and tar in addition to some other oxidants that induce oxidative stress (5). Smoking is classified as light, moderate and heavy based on the number of cigarettes consumed per day. Moderate is between 11 to 20 cigarettes, where light is < 11 and heavy is > 20 (3). On the other hand exercise is also graded as mild moderate and vigorous. Moderate intensity workout corresponds to 100 steps/min or 3000 steps/30 min which can be measured either by using pedometers or monitoring O2 uptake during exercise (6).

Based on the theoretical balancing effect between oxidants and either endogenous (SOD:superoxide dismutase or GSH-PX:Gluthatione peroxidase) or exogenous (vitamins E, C or A) antidoxidants in healthy subjects, studies have been done to show the association between malondialdehyde (MDA) levels and oxidative stress (2,7). Thiobarbituric acid reactive substances (TBARS) and MDA have been used as a biomarker of lipid peroxidation and MDA/TBARS seemed to be positively correlated with smoking (2,3). Despite studies on smoking, exercise has not been taken into account as a potential factor until now. Therefore, we aimed to investigate the acute effect of moderate exercise on oxidative stress by determining serum MDA levels in moderate smoker subjects versus non-smokers.

Material and Methods

Fifty healthy subjects performing moderate intensity exercise were assigned to 2 groups as moderate smokers smoking 11-20 cigarettes/day (Group Smoker, n=25) and non-smokers (Group Non-smoker, n=25). After obtaining ethic committee approval and consents of the participants, venous blood samples were collected to determine MDA levels half an hour before and immediately after moderate exercise according to Helsinki Declaration Rules.

Biochemical Analysis

As an indicator of lipid peroxidation MDA levels were detected in the serum by TBARS/UV as described in table 1 and calculations were made step by step accordingly (8).

Table 1. Detection and calculations for MDA analysis

	Sample	Std1 (20 nmol/mL)	Std2 (10 nmol/mL)	Std3 (5 nmol/mL)
Standard	-	250 µL	125 µL	62,5 µL
dH_2O	-	-	125 µL	187,5 µL
Sample	250 µL	-	-	-
TCA	1,25 mL	1,25 mL	1,25 mL	1,25 mL
TBA	0,5 mL	0,5 mL	0,5 mL	0,5 mL

Step 1:

- Std1 (20 nmol/mL) → 0.354
- Std2 (10 nmol/mL) → 0.186
- Std3 (5 nmol/mL) → 0.083
- 20/0.354=56.5
- 10/0.186=53.7 56.5+53.7+60.2=170.4
- 5/0.083=60.2 170.4/3=56.8 (common factor)

Step 2:

Standard curves were drawn simultaneously with analysis of study groups. Three different concentration were chosen to draw standard graphics.

Absorbance of venous blood sample was multiplied by common factor to obtain MDA:

Sample (abs/Std abs) X std concentration= MDA (nmol/mL)

Statistical Analysis

The results of the study were expressed as mean±standard deviation (sd). One way ANOVA and unpaired t-test were used to assess differences between pre-exercise and post-exercise MDA levels within smokers and non-smokers as well as between female and male subjects. A p value less than 0.05 was considered as statistically significant.

Results

There were no significant differences between moderate smokers and non-smokers with respect to demographic properties (age, BMI and gender)(Table 2).

Table 2. Demographic properties (mean±sd or n)

	Group Smoker (n=25)	Group Non-Smoker (n=25)
Age (year)	34.0±0.8	32.8±1.0
BMI (kg/cm2)	24.9±0.9	22.9±1.2
Gender (male/female)	12/13	11/14

Baseline mean pre-exercise serum MDA levels and individually either female or male subjects were comparable between smokers and non-smokers (P>0.05)(Table 3).

Table 3. Pre-exercise and postexercise serum MDA levels (nmol/mL) of female and male smoker or non-smoker subjects (mean±sd).

	Group Smoker (n=25)	Group Non-smoker (n=25)
Pre-exercise (n=25)	3.99±0.23	2.05±0.75
Female	3.99±0.33	2.09±0.34
Male	3.97±0.22	2.01±0.44
Post-exercise (n=25)	5.78±0.91*	3.25±0.88
Female	5.90±0.54#	3.20±0.65
Male	5.66±0.32	3.30±0.24

*:p<0.05 between pre-exercise vs post-exercise
#:p<0.05 between female vs male subjects

Mean post-exercise MDA levels in smokers were significantly higher than that of mean pre-exercise MDA levels of smokers (p<0.05). Additionally, post-exercise MDA levels of female smokers were significantly higher than that of post-exercise male smokers and preexercise female smokers as well (p<0.05). Whereas there were no significant differences in postexercise MDA levels of nonsmokers between males and females (Table 3).

Discussion

In the present study, oxidative stress and its relation with smoking and exercise has been demonstrated in terms of MDA. Determination of MDA as a biomarker of lipid peroxidation in serum by TBARS appears to be positively correlated with exercise in particularly female smoker subjects. As anticipated baseline pre-exercise serum MDA levels of moderate smokers in both gender were higher than that of non-smokers and post-exercise MDA levels of smokers were higher than that of non-smokers.

Analysis of MDA can be made by high performance liquid chromatography (HPLC) or TBARS. Although TBARS is a rough estimate of MDA for screening oxidative damage, it is commonly preferred because of its relative simplicity and low cost. Various clinical studies have been conducted to measure MDA levels in body fluids like saliva, urine, plasma and serum by using TBARS either UV/VIS detection (spectrophotometric) or fluorescence (FL) detection (spectrofluorometric) (9-11) (Table 4). In three of these studies, MDA levels were measured in the serum in µmol/L and they were found to be significantly higher than that of non-smokers. However, no comparison was made according to the gender. In our study when we compared serum MDA levels (in nmol/mL rather than µmol/L) between post-exercise versus pre-exercise, post-exercise MDA levels of smokers were significantly higher than that of smokers. When comparison was made with respect to gender, post-exercise serum MDA levels of female smokers were significantly higher than that of male smokers as well.

Table 4. Method of analysis of serum MDA (µmol/L) levels in smoker versus non-smokers by TBARS in clinical studies (9-11).

Total (n)	Male (n)	Female (n)	Non-smoker	Smoker	p	TBARS
123	107	14	20.7	24.0	<0.01	UV
100	50	50	1.9	2.6	<0.001	UV
71	48	23	0.22	0.37	<0.05	FL

UV: Ultra violet
FL: Fluoroescence

In contrast to significantly increased MDA levels determined by TBARS in smokers vs non-smokers in many studies, MDA levels increased in non-smoker delivering mothers in a small

cohort study (12). Therefore, our study is the 1st prospective study that shows the increased MDA in female non-smokers after moderate exercise. The reason for this conflicting result could be the powerful effect of exercise on the MDA levels.

We previously studied the possible temporal variation in antioxidant system and MDA as a lipid peroxidation biomarker in isolated erythrocytes of critically ill patients versus healthy volunteers. The MDA levels were found to be significantly higher in critically ill patients than control which was considered a sign of oxidative stress (7).

The limitation of the present study might be the lack of comparison of MDA/TBARS results with HPLC. However, MDA/TBARS is considered relevant on group basis rather than individual.

In conclusion, MDA determination in serum by TBARS/UV can be considered as a promising helpful screening tool in demonstrating the oxidative stress due to moderate exercise particularly in female smokers to reorganize a healthier life style.

Declaration of conflict of interest

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