INFLUENCE OF CIGARETTE SMOKING DURATION ON ENDOTHELIAL CELL FUNCTION

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UTICAJ DUŽINE PUŠENJA CIGARETA NA FUNKCIJU ENDOTELIJALNIH ĆELIJA

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ABSTRACT

Objective. Cigarette smoking represents an important factor for development of cardiovascular diseases. Both the amount and duration of cigarette consumption are in direct correlation to the increase in cardiovascular pathology. The aim of our research was to evaluate the influence of the cigarette smoking duration on the nitrate, nitrite, S-nitrosothiols and endothelin-1 levels in saliva of smokers.

Methods. For the purpose of this study, a total number of 60 subjects were recruited. They were divided into 4 groups based on the duration of cigarette consumption – non smokers – control (n=15); group I (n=15) – consumption of cigarettes for 2 years; group II (n=15) – 4 years and group III (n=15) – 6 years. The blood pressure, weight and height of all subjects were measured. Body mass index was calculated. Determination of nitrate, nitrite, S-nitrosothiols and endothelin-1 was done in each salivary sample collected from 60 subjects.

Results. Both systolic and diastolic blood pressure did not differ between the control group and group I. These parameters were significantly greater in group II and group III subjects compared to controls. Smokers of all groups and non-smokers had normal body mass index values. The levels of salivary nitrate, nitrite and S-nitrosothiols were significantly lower in group II and group III compared to control group. Salivary endothelin-1 concentrations were significantly higher in subjects of group II and group III than controls.

Conclusion. In this study the substantial differences between smokers and non-smokers in levels of stable metabolites of NO and endothelin-1 are due to the causal effects of smoking.

Key words: *cigarette smoking; saliva; nitrates; nitrites; S-nitrosothiols; endothelin-1; endothelium.*

SAŽETAK

Cilj. Pušenje cigareta predstavlja važan faktor za razvoj kardiovaskularnih bolesti. Količina i dužina upotrebe cigareta u direktnoj su korelaciji sa povećanjem kardiovaskularne patologije. Cilj našeg istraživanja bio je da se utvrdi uticaj dužine pušenja na nitrat, nitrit, S-nitrosotiol i nivo endothelin-1 u pljuvački pušača.

Metode. Za potrebe ove studije angažovano je ukupno 60 ispitanika, koji su podeljeni u četiri grupe na osnovu dužine korišćenja cigareta: nepušači – kontrolna grupa (n = 15); grupa I (n = 15) – koriste cigarete dve godine; grupa II (n = 15) – četiri godine i grupa III (n = 15) – šest godina. Izmereni su krvni pritisak, težina i visina svih ispitanika. Izračunat je indeks telesne mase. Određivanje nitrata, nitrita, S-nitrosotiola i endotelina-1 obavljeno je na svakom uzorku pljuvačke sakupljene od 60 ispitanika.

Rezultati. Ni sistolni ni dijastolni krvni pritisak nisu se razlikovali između kontrolne grupe i grupe I. Ovi parametri su bili znatno veći kod grupa II i III u poređenju s kontrolnom grupom. Pušači svih grupa i nepušači imali su normalne vrednosti indeksa telesne mase. Nivoi salivarnih nitrata, nitrita i S-nitrosotiola znatno su niži u grupama II i III u poređenju s kontrolnom grupom. Koncentracije salivarnog endotelina-1 bile su znatno veće kod ispitanika II i III grupe nego kod kontrolne grupe.

Zaključak. U ovoj studiji znatne razlike između pušača i nepušača u nivoima stabilnih metabolita NO i endotelina-1 posledica su uzročnih efekata pušenja.

Ključne reči: *pušenje cigareta; pljuvačka; nitrati; nitriti; S-nitrosotioli; endotelin-1; endotel.*

INTRODUCTION

Smoking is associated with a lot of adverse effects on health. Tobacco smoke contains approximately 4000 toxic chemicals including heavy metals, surfactants, cyanide, and fifty carcinogens, for example the nitrosamine compounds, nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (1, 2). Despite well-documented adverse effects, cigarettes remain one of the most popular legal consumer products used by young men and women. In 2017, 23.0% (8.2 million) of all adults in Ukraine reported current tobacco use (self-reported current use of tobacco products on a daily or less than daily basis) in any form (40.1% of males and 8.9% of females). Among ever-daily tobacco smokers aged 18-34 years, smoking was initiated on average at 16.8 years of age, and 60.4% of tobacco smokers started smoking before age 18. The average number of cigarettes smoked per day was 17.1 among daily cigarette smokers (18.2 for males and 12.6 for females) (3).

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There are numerous data have documented a relationship between smoking and cardiovascular diseases (CVD) (1, 4, 5). The majority of individuals who smoke cigarettes chronically will eventually succumb to CVD. However, despite major cardiovascular health consequences related to cigarette smoking, mechanisms of their influence on cardiovascular cell function are still being explored. The smoking of cigarettes increases the progression of atherosclerosis by an adverse effect on endothelial and smooth muscle cell functions as well as thrombotic disturbances (6-8).

The vascular endothelium plays a fundamental role in the regulation of vascular tone, thrombotic balance, growth and inflammatory responses (9). Dysfunction of these endothelium-dependent regulatory systems plays important role in the development of CVD that include hypertension and atherosclerosis. (10, 11). Among the risk factors for atherosclerosis, cigarette smoking correlates in the development of CVD (7, 11, 12). Although the precise mechanisms by which smoking could affect endothelial function are not clear, it is known cigarette smoking lead to the development of oxidative stress. During oxidative stress, the excessive generation of vascular superoxide anion and reactive oxygen species (ROS) are appeared. ROS play key role in the pathogenesis of CVD via lipid peroxidation, peroxynitrite mediated cell-damage, oxidation of low-density lipoprotein in vascular bed, activation of regulatory protein and intracellular cell transduction have been highlighted (13, 14).

Scientific evidence have been presented that tobacco-related impairment of endothelial function may be related to its adverse effects on endothelial nitric oxide (NO) synthase (15, 16). Additionally, studies report that smoke interferes with L-arginine and NO metabolism, resulting in reduced NO formation (17). Several studies have reported that the reduced NO levels may be important in smoking-induced leukocyte-endothelial interactions, at least during the early stages.

NO, nitrite (NO_2-) and nitrate (NO_3-) have gained supremacy as the available biomarkers of endothelial function. Nonetheless, no studies have been published about determination of the nitric oxide stable metabolites in combination with endothelin-1 in smokers.

Nitrites, nitrates and S-nitrosothiols have being measured traditionally in blood, urine and saliva. Saliva analysis is more acceptable noninvasive technique.

The aim of our research was to evaluate the influence of the cigarette smoking duration on the nitrate, nitrite, S-nitrosothiols and endothelin-1 levels in saliva of smokers.

PATIENTS AND METHODS

1. Subject characteristics

This research study was conducted at the Outpatient Department of Kharkiv City Student Hospital and at the Department of Biochemistry of Kharkiv National Medical University, Kharkiv, Ukraine, between January 2017 and November 2017. The study population was consisted of 60 subjects: 45 regular smokers and 15 non-smokers. Smoking behavior and lifestyle (physical activity and alcohol consumption) were recorded via a self-completed questionnaire. The information collected included: smoking status, duration of smoking (in years) and number of cigarettes smoked (per day). All subjects were assured that the results were confidential, in order to encourage accurate reporting of smoking habits. Smokers have smoked 8 - 20 cigarettes per day with tar yield 9 - 12 mg and 0.8 - 1.2 mg nicotine typical for the Ukrainian market.

The subjects were healthy young men with normal physical examination and used no medication or supplementation. Mean age of the 60 subjects was 20.9 ± 0.1 years (median 21; range 19 - 22 years).

Four groups of 60 subjects were recruited: healthy non-smoking subjects (control group, n=15); smoking healthy subjects have smoked for two years (group I, n=15); smoking healthy subjects have smoked for four years (group II, n=15); and smoking healthy subjects have smoked for six years (group III, n=15).

All individuals were placed on a nitrate-restricted diet (<15 mg per day) for 3 days before the study.

2. Blood pressure measurements

Between 8 and 10 AM, subjects were admitted in a warm (20° to 22°C) and quiet room. Blood pressure (BP) was measured using a standard cuff mercury sphygmomanometer on the left arm in a sitting position, after 10 - 15 min rest. All subjects underwent three BP measurements at either 1-min intervals. The average of the last two of three consecutive measurements was considered as the baseline BP.

3. Physical measurements

Body mass index (BMI) was calculated using the standard metric BMI formula (kg/m^2). Based on the results of calculated BMI, normal group ranged from 18.5-24.9; overweight from 25-29.9 and obese higher/equal 30.

4. Bioethics

The study was conducted in compliance with the Declaration of Helsinki and in accordance with the local Ethical Committee. All participants gave their written consent to participate in the study.

5. Collection of saliva samples

An hour prior to saliva collection, subjects were instructed to avoid eating, drinking, smoking or oral

stimulation to avoid the effect on salivary flow rate. Subjects were told to rinse their mouth with distilled water in order to remove debris and moisturize. Saliva collection was performed after 10 minutes of rest. The subjects were asked to sit in a relaxed and upright position, and saliva was allowed to drain into a test tube by gentle spitting. Saliva was collected during 15 min from 8 to 9 a.m. The five milliliters of unstimulated salivary samples were put on ice in precooled polypropylene tubes. All samples were then centrifuged at 3,000g for 10 minutes at 40°C in order to remove debris. The supernatant (upper 2/3) of each sample was fractionated into 100 μl aliquots and frozen at −60 °C until analyses (18).

6. Salivary nitrates, nitrites, S-nitrosothiols and endothelin-1 measurement

6.1. Determination of nitrate and nitrite levels in saliva

Nitrite (NO_2-) and nitrate (NO_3-) levels in saliva were determined by the method described by Miranda KM et al. (19). The sum of (NO_2-) and (NO_3-) was measured after the reduction of (NO_3-) to (NO_2-) by vanadium (III) combined with detection by the acidic Griess reaction. The (NO_3-) concentration was calculated by subtracting the (NO_2-) level from the total sum level.

6.2. Measurement of salivary S-nitrosothiols

The salivary S-nitrosothiols were measured spectrophotometrically. The principal of this method is based on formation of nitrite that are formed with Hg decay of S-nitrosothiols. Nitrites are detected using photometric strategy utilizing Griess reagent. This involves nitrite dependent diazotization of sulfanilamide, under strongly acidic conditions, followed by coupling a naphthyl ring to produce a chromophore.

6.3. Determination of endothelin-1 level in saliva

The salivary endothelin-1 level was measured using enzyme-linked immunosorbent assay kit (USA) according to the manufacturer's instructions. The optical density for each sample was measured at 450 nm using micro-ELISA plate reader (Ceres UV 900 C, BioTek Instruments Inc., Winooski, VT, USA). The endothelin-1 concentration in each saliva sample was calculated based on the standard curve.

7. Statistics

Statistical analysis of the data was carried out using the STATISTICA program (StatSoftInc., USA, version 7.0). The results were expressed as mean and standard error of mean (SEM). Differences in mean values between groups were analyzed with the Mann-Whitney U test (MW U test) for comparison of 2 groups and the Kruskal-Wallis test (KW) for comparison of 4 groups. The significance level was set at $p < 0.05$.

RESULTS

Clinical characteristics of each group are shown in Table 1. Age was well matched across all groups, taking into account the different minimum age for smoker groups and control group. The significant increase in cigarette

Table 1. Clinical characteristics of the groups in whole study population

Variables all groups (n=60)	Control group (n=15)	Group I (n=15)	Group II (n=15)	Group III (n=15)	KW
Gender (male), n	n=15	n=15	n=15	n=15	
Race (white), n	n=15	n=15	n=15	n=15	
Age (years)	20.93 ± 0.25	20.73 ± 0.18	21.07 ± 0.23	21.40 ± 0.16	NS H=5.3, p=0.1499
Duration of daily smoking (years)	0	2.39 ± 0.06	4.53 ± 0.07	6.33 ± 0.09	H=39.45, p=0.0000
Daily cigarette consumption (cigarettes/day)	0	11.20 ± 0.45	14.6 ± 0.5	17.07 ± 0.69	H=26.71, p=0.0000
BMI (kg/cm^2)	22.49 ± 0.28	22.53 ± 0.17	22.47 ± 0.18	22.81 ± 0.17	NS H=2.12, p =0.5484
Systolic blood pressure (mm Hg)	123.33 ± 0.98	125.27 ± 0.85	132.20 ± 0.59	132.87 ± 0.53	H=41.71, =0.0001
Diastolic blood pressure (mm Hg)	78.13 ± 0.95	80.07 ± 0.73	80.73± 0.56	81.40± 0.62	H=8.22, p =0.0418

Notes: Values are given as mean ± SEM; p values are for comparing multiple independent groups (Kruskal-Wallis test); NS, nonsignificant.

consumption was found in group II and group III. Group III smoked significantly more cigarettes compared to group II and group I. Duration of smoking had a positive
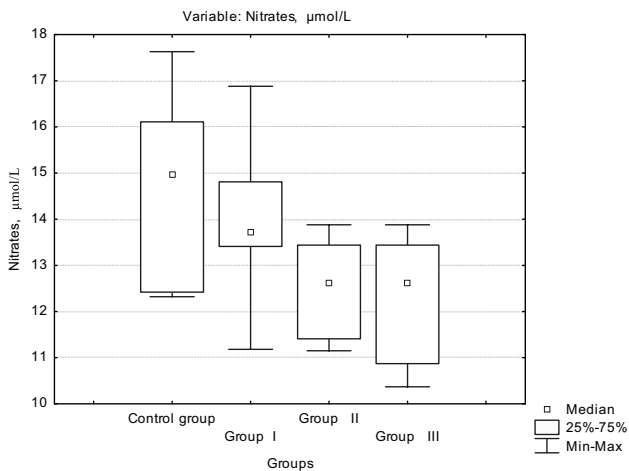
correlation with number of daily cigarettes (p = 0.0001). The number of cigarettes daily smoked by the subjects varied by duration of daily smoking: the increase of the duration of daily smoking led to elevate of the number of cigarettes smoked. Overall, systolic blood pressure and diastolic blood pressure did not differ between the control group and group I. However, the systolic blood pressure as well as diastolic blood pressure were significantly greater in group II and group III subjects as compared to controls. The mean body mass index (BMI) did not differ significantly among the groups. The salivary nitrate and nitrite levels in control group were measured 14.71±0.46 µmol/L and 2.05±0.19 µmol/L, respectively.

The concentrations of salivary nitrate and nitrite in all groups of the smokers (group I, II and III) showed significant decrease compared to the control subjects. The concentrations for salivary nitrate and nitrite were for the group I (13.92±0.37 µmol/L and 1.56±0.18 µmol/L, respectively), group II (12.50±0.24 µmol/L and 1.35±0.21 µmol/L, respectively) and group III (12.12±0.34 µmol/L and 1.06±0.16 µmol/L, respectively). The values for the

Figure 1. Salivary concentrations of nitrates in smokers and non-smokers.
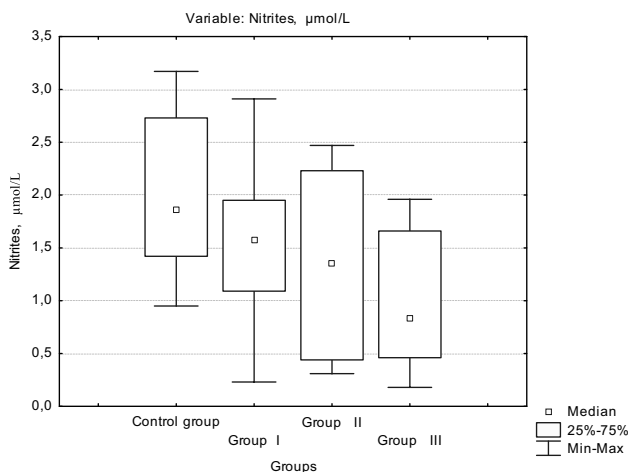
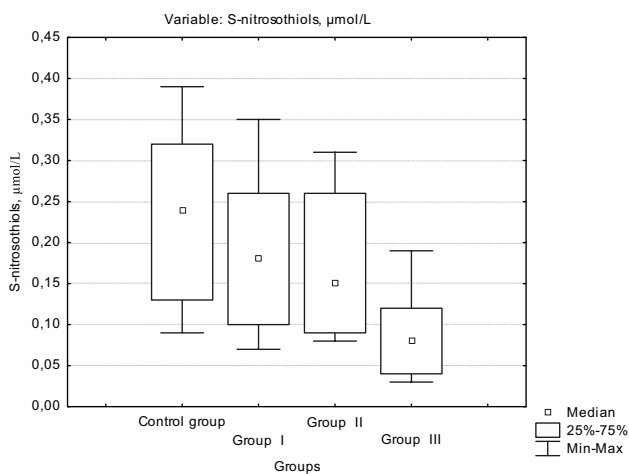*Figure 2. Salivary concentrations of nitrites in smokers and non-smokers.*

Figure 3. Salivary concentrations of S-nitrosothiols in smokers and non-smokers.
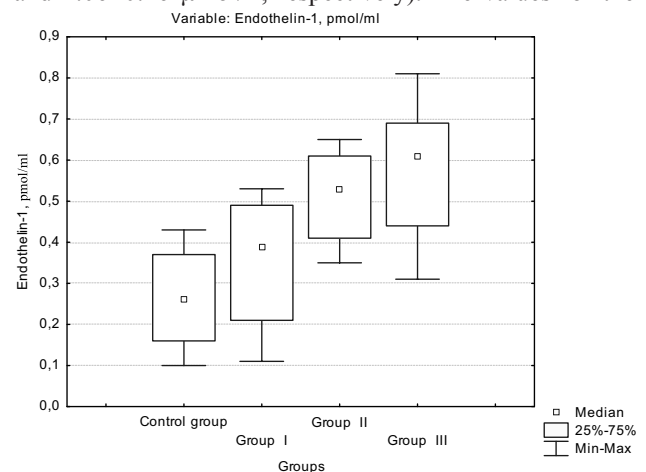

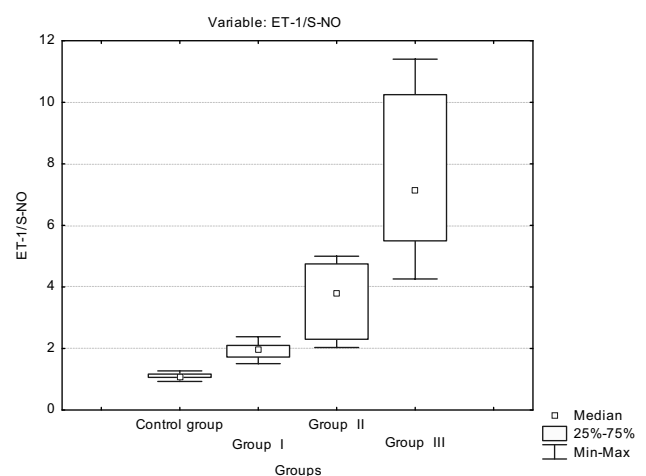*Figure 4. Salivary concentrations of endothelin-1 in smokers and non-smokers.*

Figure 5. The endothelin-1/S-nitrosothiols ratio in smokers and non-smokers.

control subjects were (salivary nitrates MW U test: ZC-I =1.56; p<0.119, ZC-II=3.21; p<0.001, ZCZC-III=3.30; p<0.001; salivary nitrites MW U test: ZCZC-I=1.68; p<0.093, ZC-II=2.24; p<0.025, ZCZC-III=3.15; p<0.016) and (nitrates KW test: H = 20.35; p = 0.000; nitrites KW test: H = 11.28; p = 0.010) (Figure 1 and Figure 2).

Significantly reduction of salivary S-nitrosothiols values were observed in group II 0.17±0.02 μmol/L (MW U test: ZC-II=2.01; p<0.044) and group III of smokers 0.09±0.01 μmol/L (ZC-III=3.67; p<0.000) compared to the values obtained in the control subjects 0.24±0.03 μmol/L. The levels of salivary S-nitrosothiols in group I didn't show significant changes compared to the control subjects. Significant difference in salivary S-nitrosothiols levels was observed in group I and group III (KW test: H = 17.71; p = 0.000) (Figure 3).

Concentration of endothelin-1 was significantly higher in group II 0.51±0.03 pmol/mL and in group III 0.58±0.04 pmol/mL compared to control group 0.26±0.03 pmol/mL and group I 0.35±0.04 pmol/mL (MW U test: ZC-II=-4.293; p<0.000, ZCZC-III=-4.231; p<0.000, ZI-II=-2.779.50; p<0.0054, ZI-III=-3.359; p<0.000) and (KW test: H = 30.38; p = 0.000). This difference was not observed between control group and group I (ZC-I =-1.804; p<0.071) (Figure 4). It should be noted, the increase of endothelin-1 concentration was not significant in smokers of group I whom had a two-year period of cigarette consumption.

These results showed a possible correlation between increased salivary endothelin-1 level and duration of daily smoking and daily cigarette consumption in smokers of group II and group III. This can be considered a result of dysmetabolic vascular changes and an increase in the production of endothelin-1, since endothelin-1 is characterized by and intensification pro-oxidant effect and vasoconstrictor activity.

The endothelin-1/S-nitrosothiols ratio was calculated and the data was presented (Figure 5). Comparison the endothelin-1/S-nitrosothiols ratio was performed between groups of smokers and control group. There was the statistically significant difference between the comparing groups. (KW test: H = 53.09; p = 0.000). There was a significant decrease in the salivary nitrates, nitrites, S-nitrosothiols levels among the group II and group III of smokers with the lowest values seen in group III.

DISCUSSION

In order to assess the endothelium functions of vessels, we studied concentration of stable metabolites of nitric oxide and endothelin-1 levels in saliva of smokers and non-smokers. The results of the study demonstrated that the content of the vasoconstrictor factor, endothelin-1, was significantly increased by 1.96- and 2.23-fold in saliva samples of group II and group III, respectively, when compared to the non-smokers of control group. At the same time, the level of S-nitrosothiols which exhibits a vasodilator effect was significantly decreased by 1.4- and 2.7- fold in saliva samples of group II and group III, respectively, with regard to the control data.

The duration of smoking and the amount of daily cigarettes consumption resulted in a statistically notable elevation of salivary endothelin-1. However, the analysis of nitrates, nitrites and S-nitrosothiols in the same subjects of group II and group III probably as suppression of antioxidant system. Thus, the results demonstrated the presence of endothelial dysfunction in group II and group III. A similar though less pronounced dynamics of this parameter was in group I. Our results are consistent with other reports suggesting an association between smoking and endothelial dysfunction, and depletion of plasma antioxidants in the body (16, 20, 21).

It has been known that endothelial dysfunction is characterized by an imbalance of the vasoconstriction and vasodilation factors, with a predominance of the vasoconstriction. The delicate balance between vasodilators and vasoconstrictors produced by the endothelium is disrupted, with disturbance in the NO pathway leading to predominance of vasoconstrictors like endothelin-1, which contribute to increase blood pressure in smokers of group II and group III (20). The present study indicates that the reduction of salivary nitrate, nitrite and S-nitrosothiols levels induced by cigarettes smoking correlated with duration of the cigarette consumption by young men of group II and group III. The prolonged cigarette consumption was associated with a decrease in the levels of stable metabolites of NO and these levels differed significantly between group I and group III of smokers.

The smoking is strong risk factor contributing toward atherogenesis and is associated with impaired endothelial function (16). The endothelium is important in maintaining vascular homeostasis and preventing the development of atherosclerosis. The perturbation of endothelium activity by cigarettes smoking led to endothelial dysfunction which increased with duration of smoking. Such endothelial dysfunction usually results in reduction of NO bioavailability (22).

The proatherogenic agents such as cigarette constituents give rise to increased free radical production (23). Salivary composition of cigarettes smokers is substantially altered with respect to free radical-involved mechanisms. Cigarette smoke contains free radicals and other oxidants in abundance. A single puff of a cigarette exposes the smoker to more than 1015 free radicals in the gas phase and additional radicals and oxidants in the tar phase (24).

Many studies have reported on the relationship between cigarette smoking and BMI. Some studies have shown that active cigarette smoking is associated with high values of BMI (25, 26), although other studies have found the opposite and, according to these data, smokers have normal BMI even less compared with non-smokers (27, 28).

Our results indicated that the BMI values were normal in smokers and non–smokers. No differences were found in mean BMI among groups. Our finding of normal BMI among smokers, overall, is consistent with a number of other studies (27, 28). Possible explanation of the normal BMI in smokers is young age of smokers and short period of cigarette consumption. In addition, nicotine is known to increase the basal metabolic rate by up to 10 % via sympathetic stimulated thermogenesis and oxidation of fatty acids (29). Nicotine can stimulate proopiomelanocortin system that lead to decrease of appetite in smokers (30).

The systolic blood pressure and diastolic blood pressure did not differ between the control group and group I. However, the systolic blood pressure as well as diastolic blood pressure were significantly higher in group II and group III subjects compared to controls. This could be explained as a result of increased vasoconstrictor effect of endothelin-1 in smokers of vasoconstrictor effect of increased endothelin-1 as consequence of smoking-related vascular changes. Endothelin-1 is linked to the pathogenesis of hypertension by means of low-grade vascular inflammation and oxidative stress at the vascular wall (31, 32). Our findings in terms of the endothelial dysfunction resulting from cigarette smoking impact are consistent with previous studies (33).

Therefore, results of this study show that cigarette smoking led to decreased stable metabolites nitric oxide (NO) and increased endothelin-1 in the salivary of smokers. The disruption of endothelium activity caused by cigarette smoking increased with duration of smoking.

We acknowledge some limitations to our study. This study was limited by age of young smokers who started smoking at age 14-18 years, so maximum duration of cigarette smoking was six years.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

LIST OF ABBREVIATIONS

BP – blood pressure

BMI – body mass index

SEM – standard error of mean

KW – Kruskal-Wallis test

MW U test – Mann-Whitney U test

CVD - cardiovascular diseases

ROS - reactive oxygen species

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PREGLEDNI ČLANAK

DIJAGNOZA HLAMIDIJALNE INFEKCIJE

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DIAGNOSIS OF CHLAMYDIAL INFECTION

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SAŽETAK

Hlamidijalna infekcija pogađa mlade seksualno aktivne osobe. Kao najčešća bakterijska seksualno prenosiva infekcija širom sveta hlamidija može da dovede do ozbiljnih posledica na reproduktivnom traktu, uključujući pelvičnu inflamatornu bolest, ektopičnu trudnoću i neplodnost sa uzromom u jajovodima. Sa druge strane, iako je kod velikog broja žena imunski odgovor sposoban da sanira infekiju, infekija može asendentno da se širi u gornji reproduktivni trakt, gde može prograđirati u perzistentnu infekiju. Dijagnostičke procedure za otkrivanje hlamidijalne infekcije uključuju direktne i indirektne metode. Lokalizovane, akutne infekcije se dijagnostikuju direktnom detekcijom patogena upotrebom ćelijske kulture, testova za kvalitativno otkrivanje antigena, testova hibridizacije i amplifikacije nukleinskih kiselina. U uslovima kada je infekcija prešla na gornji genitalni trakt, pogotovu u slučaju razvijene perzistentne infekcije dijagnoza se uglavnom postavlja indirektnim metodoma, detekcijom antitela na hlamidijalne antigene. Za dijagnozu akutne hlamidijalne infekcije neophodna je direktna detekija patogena u bolesničkom materijalu. Od svih direktnih dijagnostičkih testova, testovi amplifikacije nukleinskih kiselina su prema preporukama evropskog i američkog entra za kontrolu i prevenciju bolesti jedini testovi koji se mogu koristiti za dijagnozu akutne hlamidijalne infekcije. Ovi testovi se preporučuju zbog visoke senzitivnosti, specifičnosti i brzine dijagnostike. Za detekciju perzistentne hlamidijalne infekcije preporučuju se indirektni serološki testovi kojima se detektuje imunski odgovor, odnosno antitela specifična za hlamidijalne antigene. Naime uzorci seruma se relativno lako uzimaju, dok su uzorci tkiva sa mesta perzistentne infekcije najčešće ili teško dostupni ili nedostupni.

Ključne reči: *Chlamydia Trachomatis; dijagnoza, akutna infekcija, perzistentna infekcija.*

ABSTRACT

Chlamydial infection affects young, sexually active persons. As the most common bacterial sexually transmitted infection in the world, Chlamydia can lead to severe consequences in reproductive system, including chronic pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility. On the other hand, although in large number of women the immune response is capable of removing the pathogen, infection can ascendently spread to the upper reproductive tract where can develop into persistent infection. Diagnostic procedures for detecting chlamydial infection include direct and indirect methods. Localized acute infections are detected by direct pathogen detection using cell culture, tests for qualitative detection of antigens, hybridization tests and nucleic acid amplification tests. When the infection has passed on the upper genital tract, especially in the case of a developed persistent infection, the diagnosis is usually made by indirect methods - the detection of antibodies to chlamydial antigens. Direct pathogen detection in patient material is necessary for the diagnosis of an acute chlamydial infection. Of all direct diagnostic tests, nucleic acid amplification tests are the only tests recommended by European and American Center for Disease Control and Prevention which can be used for the diagnosis of an acute chlamydial infection. These tests are recommended for their high sensitivity, specificity and diagnostic speed. Indirect serological tests which detect immune response or antibodies specific to chlamydial antigens are recommended for the detection of persistent chlamydial infection. Serum samples are relatively easy to collect, while tissue samples from the place of persistent infection are often hard to reach or unavailable.

Key words: *Chlamydia trachomatis; diagnosis; acute infection; persistent infection*

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