International Journal of Basic & Clinical Pharmacology
Srinivasamurthy SK et al. Int J Basic Clin Pharmacol. 2023 Mar;12(2):xxx-xxx
http://www.ijbcp.com
pISSN 2319-2003 | eISSN 2279-0780

DOI: https://dx.doi.org/10.18203/2319-2003.ijbcp20230322

Original Research Article

Comparison of microvascular endothelial function as measured by laser doppler flowmeter among non-smoker and smoker males

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Received: 11 January 2023
Revised: 02 February 2023
Accepted: 03 February 2023

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ABSTRACT

Background: To understand the role of smoking in influencing endothelial function as assessed by LDF among non-smoker and smoker males.
Methods: The LDF measurement for a total of 35 non-smokers and 16 smokers was done in the central research laboratory after written informed consent. The change in LDF signal in response to acetylcholine 100 µl, which was delivered to the forearm skin by iontophoresis, was measured as perfusion units (PU).
Results: The pre-ACh LDF signal were statistically not significant between the groups. The increase in LDF signal was more prominent in non-smoker group. The LDF signal parameters such as differences in minimal response pre and post-ACh; difference in mean response pre and post ACh; the difference in maximal response pre and post ACh was not statistically significant between groups. However, the difference in the area under curve (AUC) pre and post-ACh (PU.min) (non-smoker 20089.34 (3438.92) vs smoker 13220.72 (3379.52); p=0.16) showed a trend towards statistical significance.
Conclusions: Microvascular endothelial function as assessed by LDF signal among smokers (pack-years;1.9±1.44) and non-smokers is statistically insignificant. However, lower microvascular endothelial function is observed among smokers.

Keywords: Microvascular endothelial function, Smokers, Laser doppler flowmetry, Endothelial dependent vasodilation

INTRODUCTION

Cardiovascular diseases are the leading cause of death globally.[1] Endothelial dysfunction (ED) characterized by disrupted functions such as endothelium-dependent release of nitric oxide, cell adhesion, inflammation, hemostasis, and permeability, is considered a stepping stone of atherosclerosis.[2] Hence identifying ED would pave the way for preventive strategies against atherosclerosis.[2] Factors such as age, sex, body mass index, ethnicity, hypertension, smoking status, and pollution, crosstalk with one another to influence endothelial function.[3–10] Among these tobacco smoking is of significance as it is not only well-documented risk factor for cardiovascular outcomes but preventable.[11–13] However, the extent to which smoking influence's endothelial function is still to be quantified completely.

One of the methods of assessing endothelial function is by examining the vasodilatory action of acetylcholine on the

cutaneous blood vessels detected by laser doppler flowmetry (LDF).[14,15] LDF is based on the detection of wavelength change in laser light due to moving red blood cells in the microvasculature. The photodiode measures the magnitude and frequency of wavelength shift depicting the number and velocity of moving red blood cells. Measurement of LDF signal, expressed in perfusion unit (PU) of output voltage (1 PU=10 mV) provides cutaneous microvascular blood flow with respect to time.[14,16] Iontophoresis of acetylcholine applied on the skin evokes a response by releasing nitric oxide from the endothelium, referred to as endothelium-dependent vasodilation (EDV) which is seen as an increase in LDF signal.[15] ED manifests as loss of vasodilatory response to acetylcholine.[15]

The United Arab Emirates comprises ethnically diverse populations with a diverse distribution of risk factors for cardiovascular diseases. Hence, this study is designed to understand the role of smoking in influencing endothelial function as determined by acetylcholine-induced vasodilation through LDF in male participants.

METHODS

The observational prospective study was planned and subjects were recruited using a convenient sampling method by word of mouth. The controls were males of 20 to 40 years of age who were apparently healthy, with no complaints or history of medical illnesses. The smoking subjects consisted of 20-40-year-old males with a history of smoking cigarettes for at least 1 year. Individuals with electrically sensitive systems (e.g., pacemakers), a history of any skin allergic conditions, or any history of medical illness were excluded from the study. The open Epi (Open source epidemiologic statistics for public health, version 3.0) was used for the sample size calculation of this observational study.[17] The power of 80%, alpha of 5%, and minimum difference expected between groups of 10 perfusion units were used to calculate the sample size. The allocation ratio of 1:2 was used for the smoker group and the non-smoker group, respectively. The minimum sample size was calculated to be 15 and 30, respectively, for the smoker group and the non-smoker groups. Expecting technical errors during measurement, 18 and 36 participants, respectively, for the smoker group and the non-smoker groups were intended for recruitment.

The participants were explained about the procedure, and the participant information sheet was given. All participants were instructed to abstain from tea, coffee, meal, cigarettes, and medication overnight for 12 hour before visiting the laboratory for the experiment. The participants provided their written informed consent before the experiment. The native population from Arab league countries such as United Arab Emirates, Saudi Arabia, Oman, and others of Western Asia and Northern Africa, and speaking Arabic was considered a native Arab population. Participants with origins from South Asian countries, such as India, Bangladesh, Pakistan, Sri Lanka, Nepal, Bhutan, and Maldives, were considered to be South Asians. After collecting demographic data such as age, gender, and occupation, vital parameters such as pulse and blood pressure were measured. The weight and body composition were measured using the Omron BF511 body composition monitor (Omron health care) based on the bioelectrical impedance method.

The MoorVMS laser Doppler flow (MOOR VMS-LDF2) and iontophoresis controller (MIC-2) were used to measure EDV as per the earlier reported procedure.[18–20] All participants were allowed to rest in a sitting position for 15 min with an armrest. The right forearm was cleaned using alcohol wipes. Skin probes and gel pads were applied on the volar side of the right arm. Acetylcholine was used with an anodal current to assess endothelial function. Flux 1 was connected to the iontophoresis chamber, and flux two was used as a control. The iontophoresis chamber was filled with 0.1 mL of acetylcholine solution (1 mg/mL). Acetylcholine chloride solution (ACh; Miochol-E; Novartis, stein, Switzerland) was obtained from a retail pharmacy. The working protocol for the experiment consisted of 2 min (0 mA), 10 s (25 mA), 4 min (0 mA), 10 sec (50 mA), 4 min (0 mA), 10 s (100 mA), 4 min (0 mA), 10 s (100 mA), and 8 min (0 mA) with a total duration of 22 min and 40 s. All participants were observed for 30 min before they were asked to leave the laboratory.

The protocol was approved by the ministry of health and prevention, research and ethics committee/Ras Al Khaimah subcommittee (MOHAP/RAK/SUBC/REC/2018/47-2018-F-M). The study was conducted in accordance with ICH/GCP ethics standards from April 2019 to January 2020 at the central research laboratory, Ras Al Khaimah medical and health sciences university.

Measurement of microvascular endothelial function

The perfusion in dermal capillaries, arterioles, venules, and vascular plexus representing the microcirculation in the skin was measured by LDF.[18,19] The LDF (MoorVMS-LDF2 version 5.3; Moor instruments Ltd., United Kingdom) was used to assess the microvascular endothelial response to acetylcholine 100 µl, delivered to the skin by iontophoresis (MIC2 Iontophoresis controller; Moor instruments Ltd., United Kingdom). The EDV is projected as a change in perfusion units (PUs). The LDF emits laser light at a wavelength of 780 nm and a maximum power of 2.5 mW into the skin through a probe. The LDF processor with a bandwidth between 18 Hz and 22.5 kHz was selected with a time constant of 0.1 s. The probe holder was attached to the skin using double-sided adhesive discs. The probe consists of two optical fibers: one emitting laser light and the other capturing the dermal (subsurface) backscattered doppler-shifted light. As per Doppler's principle, the frequency shift between these two lights depends on the speed of the moving red blood cells. Thus, the generated frequency shift gives the LDF signal, referred to as the blood perfusion signal, and indicates

blood flow in the volume of the illuminated tissue. The LDF output was measured as the output voltage prodperfusion unit uced per (PU).

The difference in minimal response pre and post-ACh (PU); the difference in mean response pre and post-ACh (PU); the difference in maximal response pre and post-ACh (PU); the difference in the area under the curve (AUC) pre and post-ACh (PU.min) were measured and compared among groups. The data are expressed as mean ± SD, and the significance level was set at $p<0.05$ for analytical statistics. The changes in PUs were compared between groups using an unpaired t-test. SPSS version 28.0 (IBM Corp. 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY) was used for statistical analyses.

RESULTS

A total of 67 (43 non-smokers and 24 smokers) subjects were contacted for participation in the study, as shown in Figure 1. Among them, 9 participants (4 non-smokers and five smokers) were not eligible for being out of the inclusion criteria of the age group 20-40 years. A total of seven did not wish to participate in the study (4 non-smokers and three smokers). A total of 51 subjects (35 non-smokers and 16 smokers) willingly participated in the study after giving informed.

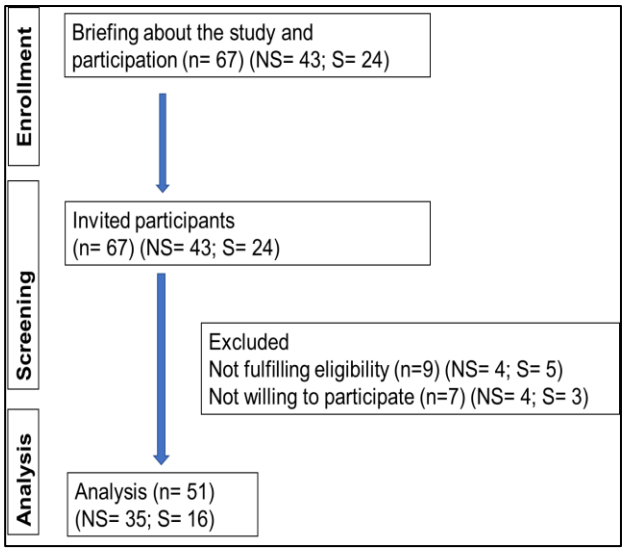

Figure 1: Flowchart of participants in the study.

The basic demographic and anthropometric characteristics of the subjects are shown in Table 1. The parameters of age, body mass index (BMI), fat percentages, muscle percentage, systolic blood pressure (SBP) and diastolic blood pressure (DBP), and heart rate did not show any statistical significance. The ethnicity of non-smokers consisted of 31 South Asian and 4 of Arab origin; and that smokers were 13 South Asians and 3 Arabs. The smoker group 'pack-years' of smoking in mean ±SD was 1.9±1.44.

Table 1: Demographics and anthropometric characteristics of participants.

Parameters		Non-smokers, (n=35) mean (SD)	Smokers, (n=16) mean (SD)
Age (Years)		32.9 (6.25)	27.5 (5.97)
Body mass index (kg/m^2)		25.7 (2.97)	26.17 (4.79)
Body composition	Fat percentage (%)	27.37 (4.57)	25.51 (7.51)
	Muscle percentage (%)	34.0 (3.37)	35.97 (3.93)
Systolic blood pressure (mmHg)		120.6 (8.97)	120 (8.07)
Diastolic blood pressure (mmHg)		78 (7.43)	76.3 (7.75)
Heart rate (per min)		72.14 (8.38)	77.68 (9.25)

The LDF measurement for all 51 participants was done in the central research laboratory with a regulated temperature of 24°C. No case of untoward events occurred during or after the procedure of LDF. The baseline LDF signal was statistically not significant. The minimal response pre-ACh (PU) (non-smoker 7.99 (1.31) vs smoker 6.12 (1.02); p=0.3); mean response pre-ACh (PU) (non-smoker 13.33 (2.92) vs smoker 9.71(1.66); p=0.4); maximal response pre- ACh (PU) (non-smoker 34.94 (13.46) vs smoker 28.25 (8.06); p=0.7); AUC pre-ACh (PU.min) (non-smoker 133.31 (29.27) vs smoker 107.18 (16.62); p=0.56) were recorded.

The increased LDF signal in response to ACh was more pronounced in the non-smoker group as compared to the smoker group. The LDF signal parameters namely differences (Δ) in minimal response pre and post ACh (PU); the difference (Δ) in mean response pre and post ACh (PU); difference (Δ) in maximal response pre and post ACh (PU); difference (Δ) in AUC pre and post ACh (PU. min) were measured and compared as shown in Table 2. The unpaired 't' test showed no statistical significance between the groups. However, the difference in AUC pre and post-ACh (PU. min) showed a trend towards statistical significance (p=0.16).

General linear model regression for confounders such as age, SBP, DBP, pulse, fat percentage, muscle percentage, ethnicity, BMI, showed no statistical significance between pack-years smoking and difference in AUC pre and post-ACh (PU. min). The correlation coefficient, [r] between pack-years and difference in AUC pre and post-ACh is -0.1 (p=0.6).

Table 2: Microvascular skin blood flow responses to acetylcholine (ACh) in non-smokers and smokers (mean ± SE).

Parameters	Non-smokers, (n=35) (%)	Smokers, (n=16) (%)
Difference in minimal response pre and post-ACh (PU)	7.98 (3.23)	4.44 (4.00)
Difference in mean response pre and post -ACh (PU)	30.17 (6.88)	19.02 (7.28)
Difference in maximal response pre and post-ACh (PU)	235.06 (47.03)	195.10 (46.12)
Difference in AUC pre and post-ACh (PU. min)	20089.34 (3438.92)	13220.72 (3379.52)

DISCUSSION

Our study showed a lower increase in LDF signal parameters among smokers as compared with non-smokers, which is not statistically significant. This finding is in the direction of several earlier studies.[21,22] Lind et al. showed impaired EDV as assessed by local infusion of methacholine and venous occlusion plethysmography among middle-aged smokers (mean age 50) but not among young smokers (mean age 25), suggesting that the duration of smoking is the key determinant.[21] Our study participants are of mean age of 30 years. Further, pack-years of smoking showed a statistically insignificant inverse relationship with a difference in AUC pre and post-ACh (r= -0.1; p=0.6). Several molecular mechanisms have been proposed for endothelial dysfunction associated with tobacco smoking, such as mitochondrial oxidative stress.[23] Further, smoking cessation reportedly improves endothelial function.[24]

Similarly, Sarabi and Lind et al showed a dose-dependent decrease in EDV as measured by forearm venous occlusion plethysmography persisting up to 30-50 min after smoking.[22] Thus indicating that both the amount and the duration of smoking to be the determinants of EDV. However, Jacobs et al showed no statistically significant changes in EDV among non-smokers and smokers.[25] Further, McVeigh et al showed similar non-significant changes in EDV between non-smoking and smoking groups.[26] The contradictory finding was observed by Rangemark et al who showed that both EDV and endothelium-independent vasodilation (EDIV) as measured by infusion of ACh and nitroprusside respectively, were larger among smokers in contrast to non-smokers.[27]

Several other studies have evaluated the relationship between smoking and EDV through ultrasound-based method of flow mediated dilation (FMD).[6,28-37] The dose dependent proportionality of smoking duration and amount to the endothelial dysfunction as assessed by FMD was also documented.[6,28,35] Long-term cigarette smoking was also documented to be associated with impaired EDV in epicardial arteries independent atherosclerotic lesions.[29] Similar impaired EDV was demonstrated in the brachial artery by FMD.[30,31]

Another randomized, open design prospective study documented immediate impaired EDV with cigar smoking.[32] Passive smoking was shown to be associated with impaired EDV to the same extent as active smoking.[33,36] Light cigarettes were shown to immediately impair brachial artery FMD to the same extent as regular cigarettes.[34] Another larger study investigated the effect of the amount and duration of tobacco smoking on endothelial function. This study showed both chronicity (≥ 40 years) and amount (≥ 30 cigarettes per day) would be associated with impaired endothelial function as measured by flow-mediated dilation (FMD).[37] However, smokers in our study have a mean smoking of 1.8 pack-years. This also could be a possible reason for the lack of statistical significance in our study.

Though several non-invasive techniques such as LDF, FMD of brachial artery, finger plethysmography, and the retinal flicker test are used to evaluate the endothelial function, none of them is considered ideal across the population.[15,38] Further, there is a strong need to generate reference values for all these techniques across the population to find clinical applications.[38] Laser Doppler flowmetry (LDF) determines EDV and EDIV by the response of endothelium to acetylcholine and nitroprusside, respectively.[15] We could not evaluate the EDIV in our study due to its explorative nature. Another limitation of our study is that diet patterns, stress levels, laboratory biochemical markers, and physical activity were not evaluated in our participants which could influence endothelial function. Though the sample size is adequate, the findings from this LDF-based method need to be confirmed with a larger sample size considering more confounding factors.

CONCLUSION

Microvascular endothelial function as assessed by LDF signal among light smokers and non-smokers is statistically insignificant. However, lower microvascular endothelial function is observed among smokers.

ACKNOWLEDGEMENTS

Authors are grateful to Dr S. Gurumadhva Rao, President, RAKMHSU for all the support during the conduct of study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Srinivasamurthy SK, Bairy LK, Naduvil SN. Comparison of microvascular endothelial function as measured by laser doppler flowmeter among non-smoker and smoker males. Int J Basic Clin Pharmacol 2023;12:xxx-xx.