Medworld –asia International Publishers
Applied Physiology and Anatomy Digest [P ISSN: 2456 – 4095]
March 2018 (2) 04, 27-33

Original article:

Impact of smoking on vascular endothelium

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ABSTRACT:

Introduction: Cardiovascular diseases are the world's largest killers. Cigarette smoking is an established risk factor for cardiovascular disease and the leading preventable cause of coronary artery disease. Endothelial dysfunction is a systemic disorder which is critical element in the pathogenesis of atherosclerotic diseases and its complications. A noninvasive method of endothelial function assessment by ultrasound technique to evaluate brachial artery called Flow– Mediated Dilatation (FMD) has emerged as a marker of endothelial dysfunction.

Objectives: To study the difference in FMD between smokers and non-smokers and to study the correlation of FMD with blood pressure (BP)between smokers and non-smokers.

Method: The present study was done on 31 smokers and 31 non-smokers (all males). Resting supine BP and brachial artery diameter was recorded by ultrasound. BP cuff was inflated 50mmHg above systolic pressure for 5 minutes and then deflated. Brachial artery diameter was again recorded for maximum dilation and FMD was calculated.

Results: FMD as an indicator of endothelial dysfunction was much less in smokers (7.33) compared to non-smokers (13.83).Systolic BP in smokers was 128.96(±8) mmHg and 119.93(±7.6) mmHg in non-smokers.

Conclusion: Significant endothelial dysfunction was observed in smokers as compared to non-smokers. Smokers were also associated with increased blood pressure which is a cause of endothelial dysfunction.

Key words: Endothelial Dysfunction, Flow Mediated Dilation, Smoking.

INTRODUCTION

Cardiovascular diseases (CVD) are the world's largest killers, claiming 17.1 million lives a year. Over 80% of CVDrelated deaths take place in low-and middle-income countries.Framingham Heart Study in 1960 found cigarette smoking to increase the risk of heart disease.[1]In 1961cholesterol level, blood pressure abnormalities were found to increase the risk of heart disease.[1]Endothelial dysfunction is a disease process that occurs throughout the vascular system and results in abnormal regulation of blood vessel tone and the loss of the atheroprotective properties of normal endothelium.[2]Assessment of endothelial function thus can provide valuable insight into pre-intrusive phase of cardiovascular disease. Brachial Artery Flow– Mediated Dilatation (FMD) described by Celermajerhas recently been much used in the study of arterial physiology.[3] Impaired brachial FMD is related to the prevalence and extent of atherosclerosis and predicts cardiovascular events.[4] FMD of the brachial artery induced by reactive hyperemia has been convincingly demonstrated to reflect systemic endothelium-dependent vasodilatory capacity, which is mediated by nitric oxide (NO) released from arterial endothelial cells.[5,6] FMD correlates with systolic blood pressure (SBP) and diastolic blood pressure (DBP) in relatively young healthy individuals and FMD of the brachial artery is known to be impaired by hypertension in middle-aged individuals.[11]Cigarette smoking is an established risk factor for cardiovascular disease and the leading preventable cause of coronary artery disease and death.[7] It reduces FMD in

asymptomatic, young adults, consistent with an early stage of endothelial dysfunction [8, 9] has been recently documented in 2007.[10]

AIMS AND OBJECTIVES

- To study Flow Mediated Dilatation among smokers and non-smokers.
- To study the correlation of Flow Mediated Dilation with Blood Pressure among smokers and non-smokers.

Endothelial dysfunction has been defined as an altered phenotype that impairs vasoreactivity or induces a surface that is thrombogenic or abnormally adhesive to inflammatory cells, is responsible, at least in part, for the initiation of thrombus formation, atherosclerosis, and the vascular lesions of hypertension and other disorders.[11]The endothelium plays a key role in the regulation of arterial tone and blood flow. In this regard, the endothelium orchestrates the production of vasodilators molecules such as NO; prostacyclin and endothelium derived hyperpolarizing factor (EDHF), and vasoconstrictors, including endothelin (ET) and angiotensin-II (AT-II). When the endothelium is dysfunctional, the vasoconstrictor effects are unopposed and arterial tone increased. In the presence of risk factors, endothelium can be activated to express adhesion molecules which are required for the adhesion of leukocytes to the endothelial surface. Endothelial expression of these factors contributes to the development of inflammation within the arterial wall and promotes atherogenesis.

Risk Factors and Endothelial Dysfunction:

Smoking:Cigarette smoking is one of the leading causes of preventable morbidity and premature mortality. It is the single most important risk factor for peripheral vascular disease and abdominal aortic aneurysm, and greatly increases the risk of stroke and heart attack.[12] However, the mechanisms by which cigarette smoking contributes to vascular disease is not yet completely understood. The major active component of cigarette smoke is nicotine.[7, 13] Nicotine has been shown to have a variety of effects on vascular biology that may contribute to atherosclerosis.At levels similar to those in the blood plasma of habitual smokers, nicotine has been shown to induce changes in expression of various atherosclerosis-related genes in endothelial cells including endothelial nitric oxide synthase, angiotensin I-converting enzyme, tissuetype plasminogen activator, platelet-derived growth factor, and basic fibroblast growth factor.[13] Nicotine has also been shown to cause morphological changes in endothelial cells, increased endothelial cell death, and enhanced transendothelial transport of plasma macromolecules.[13] However, the mechanisms of increased endothelial turnover and permeability because of nicotine have not yet been completely elucidated.

Cigarette smoking is associated with abnormal constriction of coronary arteries in young patients with angiographically normal coronary arteries[14] and in peripheral circulation. Smoking is associated with dose-dependent impairment of endothelium dependent dilatation.

Hypertension: Endothelial dysfunction occurs in the conduit artery in patients with hypertension.Several investigations have documented reduced endothelium dependent dilatation in the coronary arteriesas well as peripheral circulation in hypertensive patients.[15]

Method for clinical assessment of endothelial dysfunction:Brachial Artery FMD:

Measurement of ultrasound based FMD in the brachial artery has an intrinsic appeal, as it is noninvasive, relatively repeatable and reproducible,[3, 16] reflects important biology, has data to support its predictability, and is useful in serial studies of disease reversibility. Brachial arteryFMD is also significantly correlated with findings in the coronary circulation in the same patients.[4]FMD is currently the standard for noninvasive assessment of

conduit artery endothelial function because experience, validation, a firm biological link and association with cardiovascular events.

MATERIAL AND METHODS

Study was done on healthy males of age-group 18 - 35years from the local population of Mangalore and Kozhikode city whichincluding non-smokers and asymptomatic cigarette smokers. Study was conducted from June 2016 to June 2017 after obtaining clearance from institutional ethics committee.Inclusion criteriaincluded males aged 18-35years, non-smokers, asymptomatic smokers (regular smoking of 10 cigarettes per day for a minimum period of 1year)Exclusion Criteria includedany h/o Diabetes Mellitus,Hypertension, Alcoholics, Acute / Chronic illness,Cardiovascular disorders. Sample size - 62; 31–Smokers, 31–Non-smokers. Purposive Sampling, 95% confidence level & 90% power. Study type:Cohort - Cross Sectional Study.

Protocol of the study:

Written informed consent was taken from all the subjects who fulfilled the inclusion and exclusion criteria.Prior to the investigation detailed information was given to the participants regarding the study. Clinical evaluation, biochemical tests and assessment of brachial artery flow-mediated vasodilatation were done in all subjects.Detailed clinical examination was done which specifically included careful blood pressure recording, assessment of cardiovascular system, height and body weight measurements. Every subject was studied under similar condition with the same ultrasonography machine.Ultrasound scans were done at rest and during reactive hyperemia. Brachial artery FMD assessment was performed on all subjects using 12MHz phased array linear transducer, with high resolution Echo-Doppler PHILIPS IU22 machine. The test was done afternoon.Subjects were asked to abstain from drugs, caffeine and smoking 24hrs prior examination. Subjects were made to rest quietly in supine position for 10 minutes in temperature controlled ultrasound room before the first scan. Sphygmomanometer cuff was placed over the right arm and resting BP was recorded. Phased array linear transducer was kept proximal to the BP cuff and the right brachial artery was scanned in longitudinal section 2 to 15 cm above the ante-cubital fossa,wherever the clearest ultrasound image of the intima was obtained with aid of the edge detection software of ultrasound machine (skin overlying was marked for reference).The diameter is measured during diastole of the cardiac cycle. Systole and diastole of cardiac cycle are identified by Doppler frequency spectrum.

After the initial measurement of brachial arterial diameter, BP cuff wasinflated to atleast 50 mmHg above their SBP for exactly 5 min. During this period there was ischemia of the portion of brachial artery beneath the BP cuff and in the distal arterial segment due to complete occlusion of the brachial artery. Cuff was deflated after 5 minutes. Deflation of BP cuff produces hyperemia in the distal segment due to a transient increase in blood flow and also an increase in the brachial artery diameter.The brachial artery diameter measurements for reactive hyperemia are obtained at 45, 60, 90 seconds and 5 minutes after deflation of the BP cuff in order to measure the peak diameter due to maximal dilation of brachial artery. Diameter was measured at the same reference point and during diastole of the cardiac cycle.

FMD was calculated as- [17]

$$\text{FMD} = \frac{Maximum\ Diameter - Baseline\ Diameter}{Baseline\ Diameter} \times 100$$

RESULTS

Data analysis: Continuous variables are expressed as means ± SD. Data is analyzed by Unpaired Student's 't'Test or Chi-Square Test or Fischer's exact test as appropriate. Means were compared using Student's unpaired t-test. Pearson's correlation was used to test vicariate correlations to determine the predictors of brachial FMD for classical risk factors. Statistical significance was defined as two–sided $p < 0.05$(considered significant). Statistical analysis was performed using IBM-SPSS Ver.20 and Microsoft Office Excel 2010.

Table 1: Age(in years) comparison between Smokers and Non-smokers

Group	N	Mean	Std. Deviation	
Nonsmoker	31	24.58	4.45	t = 0.3823 p = 0.7036
Smoker	31	24.97	3.53	

Unpaired t test, p > 0.05

Table 2: Blood Pressure (mmHg) in Smokers and Non-smokers

	GROUP	N	Mean	Std. Deviation	
SBP	Nonsmoker	31	119.9354	7.6619	**t =** 4.5168 p <0.0001 VHS
	Smoker	31	128.9677	8.0781	
DBP	Nonsmoker	31	76.7742	11.73800	**t =** 2.00800 p = 0.049 sig
	Smoker	31	81.4194	5.29638	

Unpaired t test

SBP showed a very highly significant difference between the two groups whereas the DBP was just significant. Mean SBP in smokers was 128.96mmHg (± 8.07) which is higher than normal, in non-smokers it was in the normal level 119.93mmHg (± 7.66).

Table 3: Flow Mediated Vasodilation

Group	N	Mean	Std. Deviation	
Nonsmoker	31	13.83869	3.375188	**t =**8.862000
Smoker	31	7.33525	2.450257	p < 0.0001 VHS

Unpaired t test

FMD indicator of endothelial dysfunction is much less is smokers(7.33) compared to non-smokers(13.83), there is a very high difference between the two groups.

Table 4: Correlation of FMD with risk factors in Smokers

Group →		Smokers		Non- Smokers	
		SBP	**DBP**	**SBP**	**DBP**
FMD	r	-.483	-.411	-.342	-.243
	p	.006	.021	.060	.188
	N	31	31	31	31

Pearson's correlation

Correlation of FMD with risk factors in smokers showed significant correlation of FMD with SBP (*p = 0.006*), DBP (*p = 0.021*).FMD did not show significant correlation with any risk factors among non-smokers.

DISCUSSION

Cigarette smoking is an established risk factor for cardiovascular disease and the leading preventable cause of coronary artery disease and death.[7] The present study is an attempt to study the early endothelial dysfunction in smokers and to determine the association of endothelial dysfunction with risk factors of CVD.

In the present study, subjects were divided into two specified groups as smokers and non-smokers. Mean age was 24.78 ± 4.1yrs in the current study. It is found that endothelial function was significantly impaired with FMD in subjects who are smokers to be 7.33±2.41 as compared to non-smokers 13.84±3.32 (*p value < 0.0001*). This observation is in similar line to various studies where FMD is reported to be significantly lower in smokers - by Yufu K et al. (*p < 0.03*),[17] Wisemann F et al.(p = 0.03),[9] Celermajer et.al (*p < 0.001*). [18, 8]In their study Savoiu G[19] (*p < 0.001*)and Panza JA[20](*p < 0.0001*)shown that endothelial function was significantly impaired in hypertensives as compared to normotensive subjects. In the present study, endothelial function was significantly impaired in patients with higher blood pressure especially SBP*(p value 0.006*). DBP although showed an increase in smokers was significant (*p = 0.021*).

CONCLUSION

After completion of study the following conclusions were drawn.

- Endothelial dysfunction was observed in smokers as compared to non-smokers.
- Increased systolic blood pressure was associated with significant impairment in endothelial function.

Acknowledgement:

The authors acknowledge the support of Mediscan Diagnostic Centre and the Radio diagnosis department Malabar Medical College for their help in the study.

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