Original Article

Endothelial dysfunction in patients with coronary artery disease in tertiary level teaching hospital

Poudel CM, Poudyal RR, Sayami A

Department of Cardiology, Manamohan Cardiac Centre, Institute of Medicine, Maharajgunj, Nepal.

Corresponding author: Dr Chandra Mani Poudel

E-mail: chandra_poudel@yahoo.com

Abstract

Introduction: Endothelial Dysfunction is a pathological condition characterized by reduction of bioavailability of vasodilators, in particular, nitric oxide whereas endothelium-derived contracting factors are increased resulting in an impairment of endothelium- dependent vasodilation. ED can be assessed by Brachial artery flow mediated dilatation (BAFMD). The study is to determine the prevalence of endothelial dysfunction in patients with coronary artery disease.

Methods: A total of 150 adult patients above 18 years both male and female diagnosed as CAD both stable and acute were enrolled. All patients were undergone ultrasound assessment of brachial artery diameter. Brachial artery flow mediated dilatation (BAFMD) was calculated as percentage increase in diameter from the baseline and after sphygmomanometer cuff inflation. BAFMD <10% was considered abnormal, signifying ED.

Results: Overall, the prevalence of ED determined by abnormal flow mediated dilatation of brachial artery (FMD<10%) in patients with CAD was found to be 66%. The prevalence of ED in patients with acute CAD and stable CAD was found to have 62.7% and 68.7% respectively. Eight risk factors analyzed were male sex, diabetes mellitus, hypertension, smoking, family history of CAD, age, hypercholesterolemia and low high density lipoprotein (HDL). When correlating the cardiovascular risk factors with ED, diabetes mellitus (p=0.031), smoking (p=0.026), and age >45yrs in male and >55yrs in female (p=0.013) was found to be statistically significant on univariate analysis by logistic regression. When undergoing multivariate analysis, age (p= 0.012) and smoking (p= 0 .024) were significantly associated with ED.

Conclusion: Overall the prevalence of ED in patients with CAD was found to be 66%. The prevalence of ED in patients with acute CAD and stable CAD was found to have 62.7% and 68.7% respectively.

Key words: Endothelial dysfunction, Coronary artery disease Prevalence, Brachial artery flow mediated dilatation

Introduction

Endothelial Dysfunction(ED) is a pathological condition characterized mainly by reduction of bioavailability of vasodilators, in particular, nitric oxide (NO), whereas endothelium-derived contracting factors are increased resulting in an impairment of endothelium-dependent vasodilation.[1]It has been shown that ED is associated with various cardiovascular (CV) risk factors like hypertension, male sex, aging patients, hypercholesterolemia, low HDL, smoking, diabetes mellitus (DM), and family history of premature Coronary artery disease(CAD).[2,3] ED plays an important role in the development, progression, and clinical complications of atherosclerosis.[4] A study shows that 46% of patients with stable CAD and 58% of patient with acute CAD have ED.[3] In the setting of established CAD, patients with ED have higher rate

of adverse cardiovascular events compared with those with normal endothelial function.[5,6] ED is also found to be an independent predictor of future cardiac events in post myocardial infarction (MI) patients.[7] Patients whose ED persists despite treatment are at considerable risk of further events.[8] The objectives of the study is to determine the prevalence of ED in patients with CAD. This study may help to prognosticate and intensify therapy in established CAD patients (both acute and stable) who have ED.

Methods

It was a cross-sectional study conducted in the Department of Cardiology, Manamohan Cardiothoraccic Vascular and Transplant Centre (MCVTC). MCVTC, Maharajgunj Kathmandu is located in the premises of Institute of Medicine(IOM), Maharajgunj Medical Campus. It has a well equipped cardiac catheterization laboratory and non-invasive cardiac laboratory. A total of 150 adult patients above 18 years both male and female diagnosed as CAD both stable and acute were enrolled. CAD was diagnosed by Coronary angiography(CAG), either already diagnosed and admitted or admitted for CAG and diagnosed to have CAD. The study was conducted in the time span from November 2012 to October 2013. CAD was defined by CAG with > 50% stenosis in at least one coronary artery.[9]Acute coronary syndrome(ACS) patients (unstable angina, non-ST elevation MI, ST elevation MI) on CAG if found to have >50% stenosis of the coronary artery were labeled as acute CAD.ACS patients were diagnosed on clinical ground Electrocardiogram(ECG) and Troponin I.[10] Stable CAD was labeled to those patients who were admitted for elective CAG and were found to have >50% stenosis of the coronary artery or already diagnosed to have > 50% stenosis of the coronary artery by CAG and were admitted for treatment. CAG was done according to indication of the patient as per American college of cardiology/American heart association(ACC/AHA) guideline.

Inclusion criteria: All patients with age more than 18 years, both male and female who were diagnosed to have CAD both stable and acute. Exclusion criteria: Age <18 yr year, pregnant female, acute infections like- Infective Endocarditis, Urinary Tract Infection, pneumonia, anaemic patients(Hemoglobin<12gm% in female and <13gm% in male), decompensated congestive heart failure, severe Left ventricular (LV) dysfunction with (Left ventricular ejection fraction(LVEF<30%), cardiogenic shock detected on clinical ground, life-threatening arrhythmias and Atrioventricular(AV block), ventricular tachycardia, Ventricular fibrillation, uncontrolled hypertension (systolic BP >170 mmHg and diastolic BP >100 mm of Hg despite therapy), peripheral vascular disease with symptomatic claudication, absent lower limb pulses, or angiographic iliofemoral stenosis > 40%, vasculitis patients like systemic lupus erythematosus, menstruating female patient in their non-menstural phase, patient on vasoactive medication which cannot be stopped at least 12 hours before the study (such as - Nitrate, Angiotensin converting enzyme inhibitor(ACEI) and Ca channel blocker) because of instability of the patient, brachial artery with a minimum diameter (<2 mm) small arteries, patient who denied the study.

Two group of patients enrolled in the study were acute CAD and stable CAD patients. Baseline demographic data and a complete clinical history were taken from each patient. General and systemic examination of the patient was done. Drug history was taken and any drugs intake was noted. Investigations like hemoglobin, total counts, differential count, routine urine and chest X ray posterio-anterior view serum creatinine, fasting blood sugar, fasting lipid profile-total cholesterol(TC), low density lipoprotein(LDL), high density lipoprotein(HDL), triglyceride(TG), troponin I,ECG and echocardiogram was done in all patients. Fasting lipid profile was sent within 24 hrs for acute CAD patients and the following day of admission for stable CAD patients. Risk factors were determined from the basis of history and investigations. Blood pressure and heart rate were measured on the right arm in the morning before the measurement of endothelial function. Flow mediated dilatation (FMD) of the brachial artery was measured in these patients.

Patients with fasting blood glucose ≥7 mmol/L or previously established diabetes with oral antidiabetic drug or subcutaneous insulin treatment was considered diabetic according to American diabetic association 2013. Blood pressure at rest ≥140/90 mm Hg or established diagnosis with antihypertensive drug treatment was considered hypertensive according to Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypercholesterolemia was defined as fasting LDL> 4.9 mmol/L according to ACC/AHA 2013 guideline for treatment of blood cholesterol. Patients were classified as previous smokers if they had stopped smoking at

≥1 month before the study.[3] Patients who had smoked ≥1 cigarette during the last month were classified as current smokers.[3] Positive family history was present if myocardial infarction occurred in male first degree relatives age >55 years and in female first-degree relatives age >66 years, male sex was also taken as CV risk factor and HDL <1.04mmol/l in male and female <1.3mmol/L was considered as low HDL according to National cholesterol Education program(NCEP)adult treatment panel (ATP III) guideline.

Measurements of FMD

All patients were undergone ultrasound assessment of brachial artery diameter before hospital discharge according to guidelines for the ultrasound assessment of the brachial artery.[11]The test was performed with echocardiography machine Vivid 7, GE with a linear array transducer of 10 MHz frequency. Ordinary sphygmomanometer cuff was used to create occlusion of the artery.The echocardiography room was made quiet with adequate lighting and comfortable temperature. Test was performed in the morning hours after overnight fast (>6 hour). Smoking or tobacco consumption was not allowed inside the hospital as per hospital protocol. The test required no smoking or tobacco consumption for at least 6 hours prior to the procedure. Patients were not allowed to take alcohol, caffeine, cocoa, tea and fruit-juice for >12 h prior to the test. Vitamins was not allowed or stopped for at least 72 h before the procedure. Exercise was avoided >12 hr before test. All vasoactive medications were stopped for at least 12 hour prior to the procedure if possible, if not careful noting of the use of any drugs was done. Menstruating female were taken during the menstrual phase.

The test was performed after rest for at least 10 min before measurements in supine position with arm resting comfortable with the imaged artery at the heart level. A sphygmomanometer cuff was tied around the forearm (usually the dominant upper limb), approximately 1-2 cm distal to the antecubital fossa.

Once the sphygmomanometer cuff had been tied, brachial artery was imaged in longitudinal plane in the antecubital fossa. The brachial artery was scanned in longitudinal section 2 cm above the elbow, and the center of the artery was identified when the clearest picture of near-wall lumen-intima interface to far-wall lumen-intima interface was obtained. When a satisfactory transducer position was obtained, the skin was marked and the arm was kept in the same position throughout the study for reference for later measurements. Depth and gain settings were set to optimize images of interface between the lumen and the arterial wall, and the obtained images were magnified. Settings for operating the machine were not changed during the study. A resting diameter of brachial artery was first obtained. All measurements were performed at end-diastole, identified by onset of R-wave on the simultaneously acquired ECG. Average of five observations was recorded.

The sphygmomanometer cuff was then inflated to approximately 50 mmHg above systolic blood pressure and occlusion maintained for 5 minutes. After 5 minutes, cuff was deflated. Maximum arterial size, which is usually seen around 60 seconds after cuff deflation, was recorded.Averages of five observations were recorded at different time intervals. FMD was calculated as percentage increase in diameter from the baseline. Flow-mediated dilatation

= (Peak diameter – baseline diameter) / baseline diameter x 100%.

Roughly 10% increase in brachial artery diameter following cuff release is seen in healthy individuals. Less than 10% FMD was regarded as ED.

To reduce the inter-observer variability the test was conducted by single observer and to reduce the intra-observer variation five observation of brachial artery diameter before and after cuff inflation was taken and the averaged value was taken for the calculation of FMD. The intra-observer variability was also calculated. To reduce the diurnal variation the test was conducted in the morning hours.

Statistical analysis

Statistical analysis was performed with Statistical Package for social science(SPSS) version 20. For demographic profile, frequency and percentage distribution were obtained for each variable. Data were expressed as mean ± SD for continuous variables and as percentage for categorical variables. For continuous variables, differences between groups were compared with independent t-test. Chi-square test was used for nominal data. Similarly, $\chi 2$ test was used to detect linear association between the number of CV risk factors and the prevalence of ED. Univariate logistic regression analysis of risk factors was performed to estimate odds ratio and 95% confidence interval for the presence of ED. The covariates that were included in the model were CV risk factors and presence of CAD. Significant

covariates in univariate analysis were used as covariates for multivariate analysis. A two-sided P value of <0.05 was considered statistically significant.

Results

Table 1: Baseline characteristics of patients

Variables	Stable CAD	Acute CAD	P value
Age (yrs)	60.81 ± 8.72	56.88 ± 9.39	0.009
Male sex (%)	80.7	73.1	0.270
BP Systolic (mm of Hg)	121.69 ± 13.7	116.35 ± 22.53	0.077
B P Diastolic (mm of Hg)	78.39 ± 7.40	74.92 ± 7.66	0.006
Pulse rate(beats/min)	80.036 ± 14.11	85.97 ± 12.05	0.007
CV risk factors			
Hypertension**(%)**	12	24.8	0.012
Diabetes Mellitus (%)	12	29.9	0.007
Smoking **(%)**	22.9	22.4	0.941
Hypercholesterolemia (%)	6	7.2	0.759
Family history **(%)**	41	29.9	0.149
Low HDL (%)	15.7	14.9	0.901
Treatment taken			
ACEI (%)	37.3	49.3	0.143
B-blocker (%)	97.6	91	0.076
Aspirin (%)	77.6	94	0.003
Clopidogrel (%)	44.4	73.1	0.000
Statin (%)	78.3	82.1	0.565
Lab parameters			
Serum LDL(mmol/l)	2.68 ± 1.13	2.81 ± 1.17	0.501
Serum HDL(mmol/l)	0.88 ± 0.27	0.99 ± 0.29	0.018
Serum TG (mmol/l)	2.22 ± 1.377	2.022 ± 1.056	0.331
Serum creatinine (mmol/l)	93.072 ± 15.21	91.56 ± 13.19	0.524
Blood sugar fasting (mmol/l)	5.53 ± 1.88	5.9925 ± 3.16	0.270

Prevalence of ED in patients with CAD

Overall the prevalence of ED determined by abnormal flow mediated dilatation of brachial artery (FMD<10%) in patients with CAD was found to be 66%. The prevalence of ED was found to be 62.1% in male and 79.80% in female(P=065). The prevalence of endothelial dysfunction in patients with acute CAD and stable CAD was found to have 62.7% and 68.7% respectively (p=0.4).

The prevalence of ED was found to be stastistically significant on univariate regression analysis among diabetics (83.30%) as compared to non-diabetics (61.7%)(p=0.025); in smokers (82.40%)as compared to non-smokers (61.20%) (p=0.022) and in high risk age group (68.70%) as compared to low risk age group (38.90%) (P=0.010) (table 2).

The prevalence of ED was found to be statistically non-significant in patients with (68.5%) and without (64.6%) history of CAD (P=0.625);with (80%) without (65%) hypercholesterolemia (P=0.333); with low HDL (78.30%)as compared to normal HDL(68.3%) (p=0.117)and in hypertensives (79.30%)as compared to non-hypertensives (62.8%)(p=0.092)(table 2).

On multivariate analysis the prevalence of endothelial dysfunction in high risk age group (p=0.012) and smokers (p=0.024) was found to be statistically significant (table 3).

Table 2: Univariate analysis of cardiovascular risk factors as predictors of ED.

Variable	β Coefficient	Odds ratio (OR)	95% CI of OR	P-value
Male sex	-0.857	0.424	0.170-1.056	0.065
Diabetes Mellitus	1.134	3.108	1.112-8.691	0.031
Hypertension	0.820	2.270	0.859-5.994	0.098
Smoking	1.084	2.958	1.135-7.706	0.026
Family h/o CAD	0.177	1.194	0.587-2.429	0.625
Age	1.285	1.306	3.614-10.000	0.013
Hypercholesterolemia	0.767	0.440	2.154-10.540	0.344
Low HDL	0.715	2.044	0.712-5.871	0.184

Table 3: Multivariate analysis of cardiovascular risk factors predicting ED

Risk factors	β Coefficient	Odds ratio (OR)	95% CI of OR	P value
Age	1.342	3.826	1.339-10.931	0.012
Smoking	1.134	3.109	1.162-8.318	0.024

Discussion

The recorded prevalence of ED determined by abnormal flow mediated dilatation of brachial artery in patients with CAD was found to be 66% which is higher than the study done by Kalay et al, where they found 44% in ACS.[12] They used cut off value of FMD% of <7%. The reason why ED was high in the current study could be due to the high cut-off value of FMD (<10%).

But the study done by Jadhav et al found ED in patients with CAD to be 76.4% which was higher than the current study.[13] The CAD patients were documented by CAG who had AMI or ACS. ED were defined as FMD <4.5%. The reason why the prevalence is less in our current study as compared to this study could be that our study included both stable CAD and acute CAD patients who are on treatment.

When comparing the prevalence of ED in patients with stable CAD and acute CAD, it was found to be 68.7% and 62.7% respectively which was slightly higher than the study done by Toggweiler et al.[3] They found that ED defined as a PAT hyperemia ratio <1.67 in chronic CAD and acute CAD, to be 46% and 58% respectively. The reason why the prevalence is higher in our current study could be due to difference in methodology of the study.

Worsening of endothelial function in patient with ACS can be explained by oxidative stress, circulating pro-inflammatory molecules, and plaque activity.[14]

Similarly, Heitzer T et al found ED assessed by FMD predicted cardiovascular events in patients with stable CAD where they found that oxidative stress may contribute not only to ED but also to CAD activity[6]. So ED can be said to be a predictor of CV events in patients with CAD.

In the current study age (p=0.012) was independently associated with ED which was similar to study done by Kirma C et al.[15] ED had also been found in other studies in the elderly though the mechanism is not clear.[16] It has been proposed that the increase in free oxygen radicals with aging inactivates nitric oxide or has a direct toxic effect on the endothelium.[17]

In the current study smoking (p=0.024) was independently associated with ED which was similar to study done by Heitzer T et al. found blunted vasodilatory response of epicardial coronary artery diameter in the smoker as compared to non-smoker after maximal increase in coronary blood flow by Ach infusion in the presence of angiographically visible atherosclerosis by quantitative coronary angiography.[18]Celermajer found Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-

dependent arterial dilation of brachial artery in asymptomatic young adults, consistent with ED.[19] Although ED caused by smoking is multifactorial, there is sufficient evidence that free oxygen radicals have a potential role. Both passive and active smoking causes endothelial dysfunction.[19] Ozaki et al in a study found plasminogen activator inhibitor type 1 (PAI-1) and tissue plasminogen activator levels significantly ($p < 0.05$) higher in smokers compared with control subjects, could be the reason for ED in smokers. [20]

One of the findings of our study was that DM was associated with ED on univariate analysis but looses it effect on multivariate analysis. DM still continues to be an independent factor effecting endothelial function in patients with CAD. Hirai et al have shown that both acute and chronic hyperglycemia and impaired glucose tolerance cause ED.[21]In a recent study it was shown that even transient hyperglycemia induced with oral glucose in non-diabetic subjects impairs FMD.[22]Several different mechanisms have been proposed as explanation. Advanced glycosylation products extinguish nitric oxide activity in vitro and in vivo. Acceleration of the advanced glycosylation process in vivo results in a time-dependent impairment in endothelium-dependent relaxation.[23]Kirma et al in a study suggests that DM has a relationship with endothelial function in patients with CAD even when good glycemic control is achieved[15]. One of the reason why DM was not significant in multivariate analysis in this study could be that the patients enrolled had controlled fasting blood sugar 5.53±1.88 mmol/l and 5.9925 ± 3.16 mmol/l in stable and acute CAD patients.

Wallace found[24] decreased endothelial function in patients with isolated systolic hypertension compared with age-matched control subjects.However, in our study we did not find a relationship between HT and endothelial function, one of the reason could be that all the hypertensives were on antihypertensives. Antihypertensive treatment improves endothelial function, leading to less cardiac events.[25]Heitzer et al found a statistical significant difference in vasodilatory response to forearm blood flow in response to Ach between the group with hypercholesterolemia and normal subjects, blunted response with hypercholesterolemia.[26]The lack of this association in our study may be related to the characteristics of our study population because 80% were using cholesterol-lowering drugs.FMD was directly related to HDL cholesterol in a study done by Dalli E et al.[27] These studies contradict finding of our study. In our study low HDL was not associated with ED. The reason could be that our patientswere on lipid lowering drugs (80% on statins).The female preponderance of ED though not statistically significant could be due to the fact that 31 out of 34 female were ≥55 years of age and were postmenopausal. Postmenopausal women lose the protective effects of oestrogen on the vascular wall.[28]Family history of coronary artery disease was not found to be associated with ED in this study. The reason was difficult to presume. The results of statistical analysis for family history of CVD might be modified by other risk factors. Patients with a positive family history of coronary artery disease vaso constricted more as compared to those without it in a study done by Vita et al, in response to intracoronary Ach infusion.[29] The reason was suggested to be a genetic transfer of the abnormality.[30]

Conclusion

Overall, the prevalence of endothelial dysfunction determined by abnormal flow mediated dilatation of brachial artery (FMD<10%) in patients with CAD was found to be 66%.The prevalence in acute CAD and stable CAD was found to have 62.7% and 68.7% respectively.

Acknowledgements

I wish to thank all the laboratory technicians & staff of the Echo lab for providing me the much needed help in the investigation part.I would also like to thank Dr. Megha Raj Banjara for the assistance and advice on the statistical analysis part of the article.

Conflict of interest: None declared.

References

1. Davignon J and Ganz P. Role of Endothelial Dysfunction in Atherosclerosis. Circulation 2004; 109: 27-32.
2. Flammer A J, Anderson T, David S et al. The Assessment of Endothelial Function: From Research Into Clinical Practice. Circulation. 2012; 126:753-767.
3. Toggweiler S, Schoenenberger A, Urbanek N, Erne P. The Prevalence of Endothelial Dysfunction in Patients With and Without Coronary Artery Disease. Clin. Cardiol. 2010; 33(12):746–752.
4. Qasi M A, Karbanda R K, Mittal T K, Donald A E. Measurement of endothelial function and its clinical

utility. Vascular health and risk management 2008:4(3): 647-652.

5. ESC Congress. Endothelial dysfunction, inflammation and vascular reactivity. 2005; September 3-7, Stockholm, Sweden.

6. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation. 2001;104: 2673–2678

7. Erzen B, Sabovic M, Sebestjen M, Poredos P. Endothelial dysfunction, intima-media thickness, ankle-brachial pressure index, and pulse pressure in young post-myocardial infarction patients with various expressions of classical risk factors. Heart Vessels. 2007l; 22(4):215-22.

8. Kitta Y, Obata JE, Nakamura T, Hirano M et al. Persistent impairment of endothelial vasomotor function has a negative impact on outcome in patients with coronary artery disease.J Am Coll Cardiol. 2009; 53: 323–330.

9. Aksay E, Karcioglu Ö, Yantural S, Ö Kirmili. Angiographic extent of coronary artery stenosis in patients with high and intermediate likelihood of unstable angina according to likelihood classification of American Heart Association. AnadoluKardiyolDerg 2007; 7: 287-91.

10. Achar S A, Kundu S, Norcross W. Diagnosis of Acute Coronary Syndrome. Am Fam Physician. 2005; 72(01):119-126.

11. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelium-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002; 39: 257–265.

12. Kalay N, Yarlioglues M, Ardic I et al. The assessment of atherosclerosis on vascular structures in patients with acute coronary syndrome. Clin Invest Med 2010; 33 (1): E36-E43.

13. Jadhav UM, Sivaramakrishnan A, Kadam NN. Noninvasive assessment of endothelial dysfunction by brachial artery flow-mediated dilatation in prediction of coronary artery disease in Indian subjects. Indian Heart J. 2003 Jan-Feb; 55(1):44-8.

14. Kihara T, Biro S, Imamura M, et al. Repeated sauna treatment improves vascular endothelial and cardiac function in patients with chronic heart failure. J Am Coll Cardiol 2002; 39:754.

15. Kirma C, Akcakoyun M, Esen A M et al. Relationship between endothelial function and coronary risk factors in patients with stable coronary artery disease. Circ J 2007; 71: 698 –702.

16. Gryglewski RJ, Palmer RM, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature (Lond) 1986; 320: 454– 456.

17. Heitzer T, Ylä-Herttuala S, Luoma J, S Kurz et al. Cigarette Smoking Potentiates Endothelial Dysfunction of Forearm Resistance Vessels in Patients With Hypercholesterolemia: Role of Oxidized LDL. Circulation 1996; 93:1346-1353.

18. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, Deanfield JE. Cigarette smoking is associated with dose related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation. 1993; 88:2149–2155.

19. Celermajer DS, Adams MR, Clarkson P, Robinson J, McCredie R, Donald A, Deanfield JE. Passive smoking and impaired endothelium dependent arterial dilatation in healthy young adults. N Engl J Med. 1996; 334:150–154.

20. Ozaki K, Hori T , Ishibashi T, Nishio M, Aizawa Y . Effects of chronic cigarette smoking on endothelial function in young men. Journal of Cardiology 2010; 56 : 307—313

21. Hirai N, Kawano H, Hirashima O, Motoyama T, Moriyama Y, Sakamoto T, et al. Insulin resistance and endothelial dysfunction in smokers: Effects of vitamin C. Am J Physiol Heart CircPhysiol 2000; 279: 1172–1178.

22. Title LM, Cummings PM, Giddens K, Nassar BA. Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: An effect prevented by vitamins C and E. J Am Coll Cardiol 2000; 36: 2185– 2191.

23. Bucala R, Tracey KJ, Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. J Clin Invest 1991; 87: 432 – 438.

24. Wallace SM, Yasmin, Mc Eniery CM, Mäki-Petäjä KM, Booth AD, Cockcroft JR, WilkinsonIB.Isolated systolic hypertension is characterized by increased aortic stiffness and endothelial dysfunction. Hypertension. 2007; 50(1):228-33

25. Taddei S, Virdis A, Ghiadoni L, Sudano I, Salvetti A. Effects of antihypertensive drugs on endothelial dysfunction: clinical implications. Drugs. 2002; 62(2):265-84.

26. Heitzer T, Herttuala SY, Wild E, Luoma J, Drexler H. Effect of Vitamin E on Endothelial Vasodilator Function in Patients With Hypercholesterolemia, Chronic Smoking or Both. Journal of the American College of Cardiology 1999; 33(2): 499-405.

27. Dalli E, Segarra L, Ruvira J et al. Brachial artery flow-mediated dilation in healthy men, men with risk factors, and men with acute myocardial infarction. Importance of Occlusion-Cuff Position Rev EspCardiol 2002; 55(9):928-35.

28. Jensen-urstad K. &Johanson J. Gender difference in age-related changes in vascular function Journal of Internal Medicine 2001; 250: 29-36 78

29. Vita JA, Treasure CB,Nabel EG et al. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990; 81:491-497.

30. Clarkson P, Celermajer DS, Powe AJ, Donald AE, Henry RM, Deanfield JE. Endothelium-dependent dilatation is impaired in young healthy subjects with a family history of premature coronary disease. Circulation. 1997; 18; 96(10):3378-83.