

Global endothelial function assessment using pulse wave analysis in hypercholesterolemia

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To compare global endothelial function assessed by pulse wave analysis (PWA) using the ratio of endothelium dependent vasodilatation (EDV) to endothelium independent vasodilatation (EIV) in patients with hypercholesterolemia and controls. 92 subjects [46 hypercholesterolemics, 46 controls] were studied at standardized conditions. Baseline augmentation index (AIx) was assessed followed by the administration of 0.5 mg sublingual nitroglycerine, an endothelium independent vasodilator. AIx was assessed and the maximum change in AIx after nitroglycerine was recorded as EIV. After a washout period of 30 minutes, 400 µg of inhaled salbutamol, an endothelium dependent vasodilator was administered. AIx was assessed again and the maximum change in AIx after salbutamol was recorded as EDV. Global endothelial function was calculated as EDV:EIV ratio. EDV and EIV in patients with hypercholesterolemia compared to controls were 2.97 ± 3.95 and 6.65 ± 3.80 ($p < 0.001$); and 13.41 ± 4.57 and 15.88 ± 4.78 ($p = 0.01$) respectively. EDV:EIV ratio was significantly reduced in patients with hypercholesterolemia compared to controls; 0.21 ± 0.38 and 0.44 ± 0.24 ($p < 0.001$) respectively. EDV:EIV ratio was significantly reduced in patients with hypercholesterolemia compared to controls. PWA is a potential clinical tool to assess global endothelial function in patients with hypercholesterolemia.

Keywords: Endothelial function. Endothelium dependent vasodilatation. Endothelium independent vasodilatation. Augmentation index. SphygmoCor.

INTRODUCTION

Endothelial dysfunction, which is characterized by a reduction in the bioavailability of vasodilators particularly nitric oxide (NO), and a reciprocal increase in vasoconstrictors has been well implicated in the pathogenesis of atherosclerotic cardiovascular disease (Endemann, Schiffrin, 2004; Incalza *et al.*, 2018). It has been shown to precede atherosclerosis and is associated with future cardiovascular events in patients with established coronary disease (Shachinger, Britten, Zeiher,

2000). Its detection thus may serve as a useful preventive, monitoring as well as prognostic tool in cardiovascular related diseases.

The term global endothelial function refers to the measurement of endothelial function of the conduit and resistant vessels using pulse wave analysis (PWA) (McEniery *et al.*, 2006). Using the applanation tonometry technique, PWA via its parameter augmentation index (AIx) has been previously tested as a non-invasive and simple tool to assess global endothelial function (Storch *et al.*, 2017; Wilkinson *et al.*, 2002). Combining PWA with salbutamol and nitroglycerin (GTN) administration allows the assessments of endothelium-dependent and independent vasodilatations respectively; the ratio of changes in AIx to salbutamol relative to nitroglycerine indicates global endothelial function (McEniery *et al.*,

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2006). A preliminary study in patients with high total serum cholesterol demonstrated a blunted response of AIx to salbutamol (Wilkinson *et al.*, 2002) but the global endothelial function in patients with hypercholesterolemia has not been reported. This study aimed to compare global endothelial function in hypercholesterolemia patients and controls using PWA.

MATERIAL AND METHODS

This was a cross-sectional study involving 46 patients with hypercholesterolemia aged ≥ 35 years with LDL cholesterol ≥ 4.1 mmol/l being compared against 46 volunteers with normal LDL cholesterol. Source populations were the patients attending outpatient clinic during the study period. The study protocol was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (USM) and all subjects gave written informed consent according to the Declaration of Helsinki.

Pregnant subjects and subjects who were already on statins or any treatment with vasoactive medications such as ACE inhibitors and Angiotensin Receptor Blockers were excluded from the study. Before endothelial function assessment, baseline heart rate and blood pressure (BP) (Omron, England), AIx, and central arterial pressure (CAP) (PWV Limited, Australia) were assessed.

The method of assessment of endothelial function has previously been described elsewhere (McEniery, 2007; Wilkinson *et al.*, 2002). Briefly, endothelial independent vasodilatation (EIV) was induced by administering 0.5 mg sublingual nitroglycerine (Myonit Insta, Troikaa Pharmaceuticals Limited, India) for 3 minutes, after which the remaining tablet was discarded. The assessments of AIx after nitroglycerine were recorded at 3, 5, 10, 15 and 20 minutes. After a washout period of 30 minutes, endothelium dependent vasodilatation (EDV) was induced by administering 2 x 200 μ g of inhaled salbutamol (Ventolin, GlaxoSmithKline, France) via a spacer device. The assessment of AIx after salbutamol was recorded at 5, 10, 15 and 20 minutes. The maximum

changes in AIx after nitroglycerine and salbutamol were recorded as EIV and EDV respectively. A reduction in EDV: EIV ratio indicates poorer endothelial function. All AIx readings were recorded as the adjusted value at the heart rate of 75 bpm (AIx75), as AIx is known to be affected by heart rate.

Statistical analysis

Global endothelial function is defined by the ratio of AIx changes after salbutamol (EDV) relative to AIx changes after GTN (EIV). The baseline characteristics of the subjects were tested using an independent t-test and Pearson's chi-square for numerical and categorical variables respectively. The mean difference of EDV: EIV ratio between the hypercholesterolemia group and controls were tested using independent t-test and α value of 0.05 was considered statistically significant. Further analysis was done using analysis of covariance (ANCOVA) which controlled for possible confounders which are age, sex, height, body mass index, smoking status, diabetes mellitus, peripheral and central BP. Data was analyzed using IBM SPSS (Statistical Packages for Social Science) 22.0 for windows (Inc., Chicago, IL).

RESULTS AND DISCUSSION

The baseline characteristics of the subjects studied are shown in Table I. Global endothelial function represented by EDV: EIV ratio was significantly lower in hypercholesterolemic groups compared to controls; 0.21 ± 0.30 and 0.44 ± 0.24 respectively ($p < 0.001$). Similarly, EDV was also significantly lower in hypercholesterolemic groups compared to normal controls; $2.97\% \pm 3.95$ and $6.65\% \pm 3.80$ respectively ($p < 0.001$). Difference still persists after controlling for possible confounders as shown in Table II.

EIV that represents AIx response to nitroglycerine was also significantly lower in hypercholesterolemic group compared to controls; $13.41\% \pm 4.57$ and $15.88\% \pm 4.78$ respectively ($p = 0.01$).

TABLE I - Subjects characteristics

	Hypercholesterolaemia (n=46)	Control (n=46)	P value
Male/female, n/n	18/28	16/30	0.67 ^b
Age, y	48.02±8.72	45.57±7.01	0.14 ^a
BMI, kg/m ²	26.40±3.94	26.50±5.00	0.92 ^a
LDL, mmol/l	4.74±0.65	3.30±0.50	<0.001 ^a
Cholesterol, mmol/l	7.00±0.76	5.39±0.56	<0.001 ^a
Peripheral SBP, mmHg	125.28±15.34	120.60±13.36	0.11 ^a
Peripheral DBP, mmHg	76.17±11.13	74.57±9.96	0.86 ^a
Central SBP, mmHg	106.53±15.96	102.99±10.75	0.16 ^a
Central DBP, mmHg	90.49±12.18	89.19±10.23	0.59 ^a
AIx, %	27.28±7.23	26.78±8.52	0.76 ^a
EDV, %	2.97±3.95	6.65±3.80	<0.001 ^a
EIV, %	13.41±4.57	15.88±4.78	0.01 ^a
EDV:EIV ratio	0.21±0.30	0.44±0.24	<0.001 ^a
Smoking, n	3	7	0.18 ^b
Hypertension, n	6	2	0.04 ^b
Diabetes mellitus, n	3	1	0.31 ^b

BMI = body mass index; LDL = low density lipoprotein; SBP = systolic blood pressure; DBP = diastolic blood pressure; AIx = augmentation index; EDV = endothelium dependent vasodilatation; EIV = endothelium independent vasodilatation
^aIndependent t-test
^bPearson's Chi-Square

TABLE II - Comparison of global endothelial function between hypercholesterolaemia patients and healthy controls with and without adjustment of possible confounders

	Hypercholesterolemia (n=46)	Normal (n=46)	Mean difference (95% CI)	P value
EDV:EIV ratio	^a 0.21 (0.30)	^a 0.44 (0.24)	0.24 (0.09,0.39)	<0.001 ^b
	-0.06 (-0.34,0.22)	0.38 (0.16,0.60)	0.44 (0.06,0.82) ^c	<0.001 ^d

^aMean (standard deviation)^bIndicates significant difference between 2 groups using independent t-test^cAdjusted mean difference (95% confidence interval) with Bonferroni adjustment^dANCOVA applied, adjusted for age, sex, height, BMI, smoking status, diabetes mellitus, peripheral and central blood pressures

Our study showed that EDV:EIV ratio which represented global endothelial function was significantly reduced in patients with hypercholesterolemia compared to controls. To our knowledge, this was the first study that assessed EDV:EIV ratio as an index of global endothelial function in patients with hypercholesterolemia using PWA. Our recent finding was consistent with other previous reports on endothelial function in patients with hypercholesterolemia using other methods of detection such as flow-mediated dilatation (FMD) (Ikdahl *et al.*, 2015; Stapleton *et al.*, 2010) and PWA (Wilkinson *et al.*, 2002). An earlier study by Wilkinson *et al.* (2002) reported a reduced response of AIx to salbutamol in hypercholesterolemia. In our study, this was represented by EDV and similarly it was significantly reduced in patients with hypercholesterolemia compared to controls. Several versions of endothelial function assessments by PWA were described by different studies, but all methods were based on the waveform changes in response to salbutamol and nitroglycerine (Lekakis *et al.*, 2011). Whilst the earlier studies defined global endothelial function as salbutamol-induced changes in AIx as represented by EDV (Hayward *et al.*, 2002; Wilkinson *et al.*, 2002), the later studies described endothelial function as EDV:EIV ratio (McEniery, 2007; McEniery *et al.*, 2006). McEniery *et al.* (2006) described global endothelial function as the ratio of AIx change to salbutamol relative to nitroglycerine whereby a reduction in the ratio indicates worse endothelial function. In their study, global endothelial function in healthy population was described (McEniery *et al.*, 2006). Studies in patients with risk factors and cardiovascular-related disease are still lacking and ours was the first study to describe the ratio in patients with hypercholesterolemia. Compared to our cohort of hypercholesterolemia, global endothelial function, as described by McEniery *et al.* (2006) appeared among a healthy population.

When used as a control, AIx is expected to remain unchanged after nitroglycerine stimulation. However, in our study, hypercholesterolemia group showed a reduced response to nitroglycerine. While a few previous studies reported no difference in AIx

after nitroglycerine stimulation in healthy volunteers (Hayward *et al.*, 2002; Ibrahim *et al.*, 2009; McEniery *et al.*, 2006) and hypercholesterolemic cohort (Wilkinson *et al.*, 2002), a few recent studies have also reported a reduced response in AIx to nitroglycerine in different medical conditions. Using the same method of detection of PWA, Covic *et al.* (2004) reported a reduced response of AIx to nitroglycerine in chronic kidney disease (CKD) patients and the response improved after dialysis. Similarly, Paul *et al.* (2009) also reported a reduced AIx response to nitroglycerine in patients with chronic heart failure. Compared to hypercholesterolemia patients with no concomitant disease in the preliminary study reported by Wilkinson *et al.* (2002), our cohort of hypercholesterolemia patients were documented to have other concurrent medical conditions which include hypertension (6 patients) and diabetes mellitus (three patients). Other studies assessing endothelial function via other methods such as FMD also reported impaired EIV by nitroglycerine in subjects with cardiovascular risk factors and coronary heart disease (Maruhashi *et al.*, 2013; Raitakari *et al.*, 2001). It has been reported that in cardiovascular related diseases, an increased presence of superoxide has been detected (Maruhashi *et al.*, 2013). Superoxide, other than inhibiting NO production by endothelium has also been shown to inhibit intravascular signaling in vascular smooth muscle cell by inhibiting guanylyl cyclase and cGMP dependent kinase which may result in smooth muscle cell dysfunction. It has also been suggested that a reduced response to nitroglycerine was correlated with increased intima-media thickness (Maruhashi *et al.*, 2013) and other possible structural abnormalities such as increased left ventricular mass (Covic *et al.*, 2004). It was thus suggested that global endothelial function should be interpreted as an index of both EDV and EIV, rather than solely by EDV particularly in high risk patients with cardiovascular related disease.

This study has demonstrated that global endothelial function assessed by PWA combined with pharmacological challenge was reduced in hypercholesterolemia patients. Global endothelial function should be interpreted as the ratio of EDV:EIV particularly in high risk patients with cardiovascular

related disease. Further studies should include testing its application in monitoring treatment before it could be widely used as a tool to assess endothelial function.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

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