Ind J Clin Biochem (Apr-June 2020) 35(2):245–250
https://doi.org/10.1007/s12291-019-00861-w

SHORT COMMUNICATION

Endothelial Microparticle as an early Marker of Endothelial Dysfunction in Patients with Essential Hypertension: A Pilot Study

Santhi Silambanan[1] · R. S. Hermes[1] · Emmanuel Bhaskar[2] · Sri Gayathri[3]

Received: 19 February 2019 / Accepted: 16 November 2019 / Published online: 28 November 2019

Abstract Hypertension is a global health burden causing immense morbidity and mortality especially from the complications of end-organ damage. It is expected to affect 29% of the population by the year 2025. Hypertension is usually asymptomatic; it is diagnosed by a disease of exclusion. Numerous factors such as inflammation, oxidative stress, genetic predisposition etc. play roles in the pathogenesis of hypertension. Endothelial microparticles (EMPs) are released into the circulation with the onset of changes in endothelium, even before the release of other routine vascular endothelial markers. EMPs mediate inflammation, thrombosis and vasoconstriction of blood vessels in hypertensives. This pilot study was undertaken to assess whether EMPs are early markers of endothelial dysfunction in essential hypertensive patients. The study was conducted as a large case control study in which 525 individuals were involved. It consisted of three study groups: Group I: individuals with normal blood pressure (JNC8), group II: hypertensives without evidence of end-organ damage and group III: hypertensives with evidence of end-organ damage. Homocysteine, hsCRP, fibrinogen, eNOS, oxLDL and other markers were measured. For analysis of EMPs a subset of individuals are taken from each group. Control group of 10 individuals who had homocysteine level more than15μmol/L was taken as Group I. Another 10 individuals were taken randomly of five each from groups II and III. EMPs were analyzed by flow cytometry and were identified as CD31 +, CD42 − microparticles with diameters < 1.0 mm. There was significant increase in EMPs ($p = 0.035$) in hypertensive individuals with end organ damage. Measurement of EMPs in hypertensive individuals could help physicians in identifying and initiating therapeutic interventions at a very early stage of the disease, thus improving the quality of life.

Keywords Hypertension · Biomarkers of endothelium · Endothelial dysfunction · Endothelial microparticles · Flow cytometry · Homocysteine · Atherosclerosis · Endothelial injury · Endorgan damage

Abbreviations

EMPs	Endothelial microparticles
eNOS	Endothelial nitric oxide synthase
OxLDL	Oxidized low density lipoprotein
hsCRP	High sensitive C-reactive protein
MFI	Median fluorescence intensity
TF	Tissue factor
TNFα	Tumor necrosis factor alpha
PAI-1	Plasminogen activator inhibitor-1
LPS	Lipopolysaccharide
ROS	Reactive oxygen species

✉ Santhi Silambanan
santhisilambanan@gmail.com

R. S. Hermes
rs.hermes73@gmail.com

Emmanuel Bhaskar
drebhaskar@gmail.com

Sri Gayathri
ssriga@yahoo.com

[1] Department of Biochemistry, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Porur, Chennai 600116, India

[2] Department of General Medicine, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai 600116, India

[3] Department of Pathology, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai 600116, India

Introduction

Essential Hypertension is a chronic multifactorial disorder predisposing to functional and structural alterations of the arteries. This leads to arterial stiffening and vasomotor tone abnormalities leading to end-organ damage. Various pathogenic mechanisms such as inflammation, oxidative stress, environmental insults and genetic predispositions lead to endothelial injury and dysfunction. Endothelial dysfunction is a systemic disorder affecting entire endothelium of blood vessels of the body with resultant atherosclerosis and its complications. EMPs constitute 5–15% of all microparticles in blood circulation. EMPs are considered to be released from endothelial cells during activation, apoptosis or injury of endothelial cells. They cause damage to the blood vessels by initiating inflammation, thrombosis and angiogenesis [1]. EMPs are complex vesicular structures ranging in size from 0.1 to 1.0 μm. EMPs are found to be the sole predictors of adverse events in patients with cardiovascular diseases. Commonly used anti-hypertensive agents such as beta-blockers, angiotensin receptor blockers and calcium channel blockers are found to decrease the level of EMPs. This study was undertaken to find out whether EMPs are early markers of endothelial dysfunction in hypertensive individuals.

Materials and methods

This study was initiated with a large case control study with participants consisting of 175 in each group; group I consisted of individuals with normal blood pressure as per JNC criteria 8, Group II consisted of hypertensives without evidence of end-organ damage and Group III consisted of hypertensives with features of end- organ damage. All the participants were selected from the patients attending General Medicine out-patient department in Sri ramachandra Medical College and Research Institute. Individuals who had normal blood pressure and were attending the outpatient department for other ailments were taken as the control group (group I). Hypertensive individuals were grouped into groups II and III, depending on the presence or absence of evidence of end-organ damage. End-organ damage in hypertensives were assessed by either of the following features such as changes in heart mass measured by ECHO and ECG, retinopathy by fundus examination, renal features by measurement of serum creatinine > 1.2 mg/dl and presence of microalbuminuria. Group II individuals did not have features of end-organ damage; while individuals in group III had features of end-organ damage. The study included individuals of both gender in the age group of 20–55 years. Individuals on drugs such as steroids, oral contraceptive pills and fibric acid derivatives were excluded from the study. Homocysteine was measured by EIA (AXIS Shield), high sensitive C-Reactive Protein (hsCRP) by Turbidimetric Immunoassay method (Quantia CRP), fibrinogen by Turbidimetric immunoassay method (Quantia® Fibrinogen), eNOS by ELISA (Bioassay laboratory technology) and Oxidized LDL (OxLDL) by ELISA (R&D SYSTEMS Minneapolis, USA).

Analysis of endothelial microparticles (EMPs)

Worldwide a homocysteine level of more than 15μmol/L was considered as hyperhomocysteinemia which can lead to cardiac and coagulation disorders. 10 individuals from group I who had homocysteine level of more than 15μmol/L were chosen as the control group. 5 individuals from group II and another 5 individuals from group III were chosen for analysis of EMPs. This sample size of 20 is decided due to cost constraints. EMPs were analyzed by flow cytometry in BD FACS CALIBUR (BD Biosciences) in the methodology described byYongguang Lu et al. [2]. Citrated blood samples were centrifuged at 160 g for 10 min to obtain platelet rich plasma. This was again centrifuged at 1500 g for 6 min to produce platelet poor plasma (PPP). 50μl of PPP was mixed with ant-CD31-PE and anti-CD42-FITC (BD Biosciences, USA). After incubating at room temperature, pellets were suspended in 1 ml of phosphate buffer solution and the mixture was subjected to flow cytometry. EMPs were identified as CD31 +, CD42 − microparticles with diameters < 1.0μm [3]. EMPs express many membrane surface antigens, including CD31, CD51 and CD62E compared with membrane microparticles derived from other cell types and CD31 is a more sensitive marker of vascular injury than CD51 [4, 5]. Since platelets microparticles (PMPs) also express CD31, CD42b, the same was used to exclude the contribution of platelets in microparticle formation. In this study, EMPs were defined as cells having CD31 +/CD42 − surface antigens [6]. Analysis of EMPs was performed using forward scatter (FSC) or side scatter (SSC) [7].

Quantifying EMPs: Usually the samples are spiked with a known amount of commercially available counting beads and the concentrations of EMPs are calculated and as expressed as number of EMPs/μL. In the present study counting beads were not used due to paucity of funds [7]. EMPs were calculated as percentage of CD31 +/CD42 − particles (EMPs) against the total of CD31 +/CD42 + and CD31 +/CD42 − (total of PMPs and EMPs). Median Fluorescence Intensity (MFI) was also measured.

Ethics clearance was obtained from Institutional Ethics Committee (IEC-NI/14/JUN/40/35). Written informed consent was obtained from the participants before being enrolled in the study. Statistical analyses were performed with SPSS software of version 13.0.

Results

There was significant difference between the groups in homocysteine, hsCRP, fibrinogen, eNOS and oxLDL as shown in Table 1. Tukey post hoc test showed statistical significant in all the endothelial markers between the three groups.

Analysis of endothelial microparticles

When EMPs were compared with endothelial markers in the three groups, EMPs showed positive correlation with postprandial glucose (p value = 0.049) and negative correlation with uric acid (p value = 0.048) in group I; negative correlation with eNOS (p value = 0.042) in group II and positive correlation with fasting glucose (p value = 0.049) and negative correlation with uric acid (p value = 0.027) in Group III. Also data from groups II and III were combined as the case group and compared with group I (control). There was found be positive correlation with systolic blood pressure (p value = 0.0493), postprandial glucose (p value = 0.04), LDLc (p value = 0.025), creatinine (p value = 0.047) and negative correlation with uric acid (p value = 0.014).

Discussion

Hypertension is a chronic metabolic disorder involving the endothelium of the arteries. Endothelium releases biomarkers which have vasodilatory effect thus lowering vascular resistance. The function of the endothelium gets disrupted in the presence of inflammation, oxidative stress and other environmental toxins. This predisposes to endothelial injury, activation, apoptosis or fibrosis. If the injury is mild and localized, endothelium can revert back to normal by the process of remodelling. Usually the precipitating factors are long standing and cannot be controlled as well as they are not self-limiting. Hence this leads to continuing endothelial injury resulting in endothelial dysfunction along with onset of incipient end-organ damage. Thus, endothelial dysfunction represents the earliest event in essential hypertension [8]. EMPs levels in circulation are a balance between formation and clearance [9]. In patients who are predisposed to hypertension, formation of EMPs is more than its clearance. Homocysteine with a blood level of more than15μmol/L is known to cause endothelial dysfunction with resultant shedding of microparticles from the endothelial cells. This is prominent in situations when there is increased tissue factor (TF) expression and procoagulant activity [10]. EMPs formation are found to be induced by thrombin, angiotensin II, modified lipids, tumor necrosis factor-alpha (TNFα), plasminogen activator inhibitor-1 (PAI-1), bacterial lipopolysaccharides (LPS) and uremic toxins [11].

The study participants were grouped into three based on the presence or absence of hypertension and its complications. 40 percent were male participants in all the three groups. 80% of the study participants were nonvegetarians. Smokers and alcoholics constituted 40 percent. Fasting glucose, postprandial glucose, total cholesterol, HDLc, triglycerides and creatinine were within the reference interval in all the groups except LDL in group III, but it was not statistically significant (p value = 0.082). Microalbumin and albumin creatinine ratio showed statistical significant difference of p value = 0.0009 and p value = 0.006 between the groups respectively. They were markedly elevated in group III which consisted of hypertensives with features of end-organ damage.

The levels of the endothelial markers were proportionately altered from group I to group III (Table 1). There was significant increase in homocysteine (p value = 0.0001), hsCRP (p value = 0.0002), fibrinogen (p value = 0.0001), oxidized LDL (p value = 0.0001), and significant decrease in eNOS (p value = 0.0001). There was direct relationship between inflammatory, oxidative stress and procoagulant markers and inverse relationship with eNOS which catalyzes formation of the vasodilator, nitric oxide (NO). According to Karaouzeneet al, inflammatory and pro-

Table 1 Levels of endothelial markers

Parameters	Group I (n = 10)	Group II (n = 5)	Group III (n = 5)	Significance
Homocysteine (μmol/L)	12.85 ± 2.56	30.0 ± 9.82	40.2 ± 5.54	p = 0.0001***
hsCRP (mg/L)	1.24 ± 0.93	2.78 ± 1.16	4.72 ± 1.54	p = 0.0002***
Fibrinogen (mg/dL)	58.5 ± 21.56	122.0 ± 48.84	297.4 ± 97.44	p = 0.0001***
eNOS (pg/mL)	634.7 ± 56.28	591.0 ± 55.38	205.6 ± 66.39	p = 0.0001***
Oxidized LDL (ng/dL)	352.0 ± 114.46	582.2 ± 53.48	783.6 ± 192.86	p = 0.0001***

(Expressed as mean ± SD); p value *significant, **highly significant, ***very highly significant

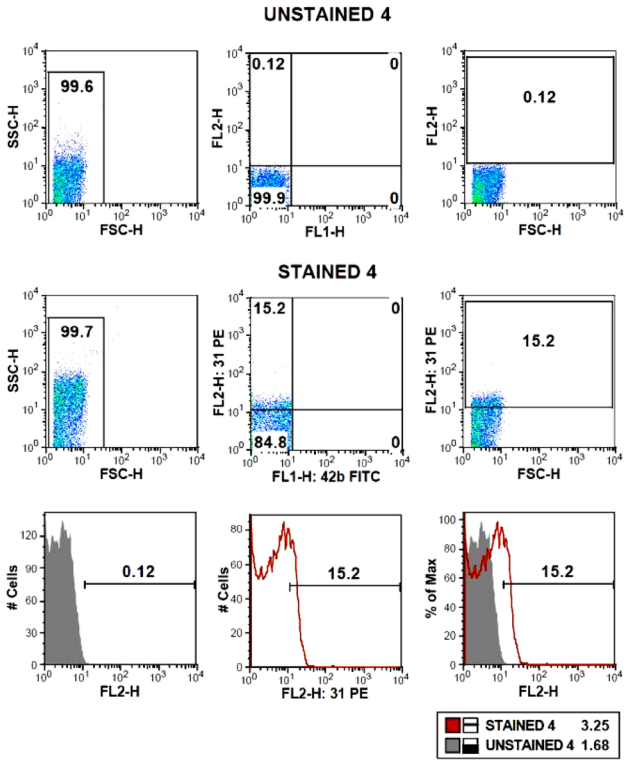

Fig. 1 EMPs in hypertensives with end organ damage (Group III)

oxidant markers are elevated with decrease in antioxidant markers in obese women with hypertension [12].

In this study, percentage of EMPs in group III with a value of 13.51 ± 4.38 and Median Fluorescence Intensity (MFI) of 3.0 ± 0.19 was the highest in hypertensive patients with end organ damage (Fig. 1) (Table 2). Post-hoc test shows that there is significant increase in group II compared to group I (p value = 0.002). But there was no statistical significant difference between group II and III (p value = 0.8339). This is probably the levels of EMPs are increased to higher magnitude in hypertensives even before the clinical evidence of end-organ damage. Also the participants in group II could have end-organ damage which were not identified by the routine examinations. It was found that CD31 +/CD42 − EMPs were more frequently expressed in acute conditions [13, 14]. Richard A Preston et al. have shown that EMPs CD31 + were released due to microvascular endothelial apoptosis [2]. According to Jemenez et al., EMP numbers are said to be inversely correlated with vasodilatation [5].

In this study, EMPs were compared with other endothelial markers and it showed positive correlation with fasting and postprandial glucose and negative correlation with uric acid and eNOS. Groups II and III together (cases) when compared with group I (control) there was found be positive correlation with systolic blood pressure (p value = 0.0493), postprandial glucose (p value = 0.04), LDLc (p value = 0.025), creatinine (p value = 0.047) and negative correlation with uric acid (p value = 0.014). EMPs contribute to both initiation and progression of cardiovascular diseases by stimulating foam cell formation and increasing endothelial activation and platelet aggregation [15]. EMP numbers are positively correlated with interleukin-6 and hsCRP indicating chronic inflammation playing a role in the release of EMPs. EMPs cause negative effects on endothelium through modification of nitric oxide production [16, 17]. This is probably due to decreased phosphorylation of serine residues on eNOS. CRP also influences EMP formation through effects on eNOS [18]. Increased oxidative stress has been associated with increased circulating level of microparticles. According to Szotowski et al., there is positive relationship between reactive oxygen species (ROS) and TF of EMP origin [19]. Early diagnosis of endothelial dysfunction is mandatory to expose the individuals to the anti-inflammatory and endothelium-protective agents at an early stage of the disease. Endothelial microparticles by reflecting endothelial dysfunction may act as a valuable tool in assessing endothelial dysfunction, particularly in asymptomatic individuals [20].

Table 2 (a) Showing analysis EMPs in the three groups. (b) Tukey Post hoc test

Parameters	Group I (n = 10)	Group II (n = 5)	Group III (n = 5)	Significance p value
(a)				
% of EMP	8.44 ± 3.0	10.51 ± 2.37	13.51 ± 4.38	0.035*
Median fluorescence intensity (MFI)	2.4 ± 0.16	2.93 ± 0.25	3.0 ± 0.19	< 0.0001***
Parameters	Group I versus Group II p value	Group I versus Group III p value	Group II versus Group III p value	
(b)				
% of EMPs	0.002**	0.001***	0.8339	
Median fluorescence intensity (MFI)	0.49	0.0304*	0.3421	

(Expressed as mean ± SD); p value *significant, **highly significant, ***very highly significant

Conclusion

EMPs are elevated in patients with hypertension before the onset of end-organ damage. EMPs are positively correlated with inflammatory and oxidative markers such as oxLDL and hsCRP and negatively correlated with vascular dilator marker, eNOS. The increasing level of EMPs in hypertensives predicts the severity of endothelial dysfunction. Thus measurement of EMPs will give promising results in taking adequate measures to prevent as well as delay the progress of hypertension. This decreases mortality and morbidity thus reducing the wastage of individual's and society's economy.

Limitations

This study on endothelial microparticles is done in a small sample size. It is done as a case–control study and hence cause and effect relationship could not be elicited. The study can be extended to find out the association of plasma homocysteine level and EMPs level. It is essential to bring improvements in the methodology of EMPs by incorporation of counting beads to increase the accuracy of EMPs results.

Acknowledgements An oral presentation was done titled "Endothelial Microparticles as a marker of Endothelial Dysfunction in patients with Essential Hypertension" in AMBICON 2017—National conference held at Mysuru, by the Association of Medical Biochemists of India, from 17-11-2017 to 19-11-2017. The authors acknowledge Dr. Sathiasekaran, Professor in Community Medicine for his contribution in designing the study and analyzing the results.

Authors' contributions Hermes analyzed the samples for estimating biomarkers, Sri Gayathri helped in assessing endothelial microparticles by flow cytometry, Santhi Silambanan and Emmanuel Bhaskar were involved in writing the manuscript. All authors read and approved the final manuscript.

Funding The work has been partly funded by Shri NPV Ramasamy Udayar Chancellor Research Fellowship. This fund was utilized for analysis of biological samples obtained from the participants.

Availability of data and material The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest Santhi Silambanan was the brain behind this research and guided Hermes in conducting this research, which is in continuation of her Ph.D. work. Hermes has received the fund Shri NPV Ramasamy Udayar Chancellor Research Fellowship which supported part of this research. Emmanuel Bhaskar was a member of the research advisory committee, whose advice contributed to the results and discussion of the article. Sri Gayathri helped in doing the analysis of EMPs by flow cytometry in Central Research Facility of our Institute. The authors declare that they have no conflict of interest.

Ethics approval and consent to participate This study has been carried out in accordance with the Declaration of Helsinki and ethics clearance was obtained from Institutional Ethics Committee (IEC-NI/14/JUN/40/35). Written informed consent was obtained from the participants before being enrolled in the study. Only participants who volunteered to take part in the study were included.

References

1. Ivak P, Pitha J, Netuka I. Circulating microparticles as a predictor of vascular properties in patients on mechanical circulatory support; hype or hope? Physiol Res. 2016;65:727–35.
2. Lu Y, Li L, Yan HY, Su Q, Huang J, Fu C. Endothelial microparticles exert differential effects on functions of Th1 in patients with acute coronary syndrome. Int J Cardiol. 2013;168:5396–404.
3. Preston RA, Jy W, Jimenez JJ, Mauro LM, Horstman LL, Valle M, et al. Effects of severe hypertension on endothelial and platelet microparticles. Hypertension. 2003;41:211–7.
4. Lechner D, Kollars M, Gleiss A, Kyrle PA, Weltermann A. Chemotherapy-induced thrombin generation via procoagulant endothelial microparticles is independent of tissue factor activity. J Thromb Haemost. 2007;5:2445–52.
5. Jy W, Jimenez JJ, Mauro LM, Horstman LL, Cheng P, Ahn ER, et al. Endothelial microparticles induce formation of platelet aggregates via a von Willebrand factor/ristocetin dependent pathway, rendering them resistant to dissociation. J Thromb Haemost. 2005;3:1301–8.
6. Liaw PC, Neuenschwander PF, Smirnov MD, Esmon CT. Mechanisms by which solubleendothelial cell protein C receptor modulates protein C and activated protein C function. J Biol Chem. 2000;275:5447–52.
7. Alkhatatbeh MJ, Enjeti AK, Baqar S, Ekinci E, Liu D, Thorne RF, Lincz LF. Strategies for enumeration of circulating microvesicles on a conventional flow cytometer: counting beads and scatter parameters. J Circ Biomark. 2018;7:1–10.
8. Bartoloni E, Alunno A, Bistoni O, Caterbi S, Luccioli F, Santoboni G, et al. Characterization of circulating endothelial microparticles and endothelial progenitor cells in primary Sjögren's syndrome: new markers of chronic endothelial damage? Rheumatology. 2015;54:536–44.
9. Dignat-George F, Boulanger CM. The many faces of endothelial microparticles. Arterioscler Thromb Vasc Biol. 2011;31:27–33.
10. Sekula M, Janawa G, Stankiewicz E, Stepien E. Endothelial microparticle formation in moderate concentrations of homocysteine and methionine in vitro. Cell Mol Biol Lett. 2011;16:69–78.
11. Curtis AM, Edelberg J, Jonas R, Rogers WT, Moore JS, Syed W, et al. Endothelial microparticles: sophisticated vesicles modulating vascular function. Vasc Med. 2013;18:204–14.
12. Karaouzene N, Merzouk H, Merzouk AS, Bouanane S, Loudjedi L, Merzouk SA. Interrelations between inflammatory and oxidative stress biomarkers in obese women with two complications (hypertension, diabetes). Rom J Diab Nutr Metab Dis. 2019;26(2):129–43.
13. França CN, Izar MCO, Amaral JB, Tegani DM, Fonseca FAH. Microparticles as potential biomarkers of cardiovascular disease. Arq Bras Cardiol. 2015;104:169–74.
14. Schiro A, Wilkinson FL, Weston R, Smyth JV, Serracino-Inglott F, Alexander MY, et al. Endothelial microparticles as conveyors of information in atherosclerotic disease. Atherosclerosis. 2014;234:295–302.

15. Berezin AE. Impaired phenotype of circulating endothelial-derived microparticles: novel marker of cardiovascular risk. J Cardiol Ther. 2015;2:365–70.
16. Devaraj S, Kumaresan PR, Jialal I. C-reactive protein induces release of both endothelial microparticles and circulating endothelial cells in vitro and in vivo: further evidence of endothelial dysfunction. Clin Chem. 2011;57:1757–61.
17. Mikirova N, Casciari J, Hunninghake R, Riordan N. Increased level of circulating endothelial microparticles and cardiovascular risk factors. J Clin Exp Cardiol. 2011;2:2–4.
18. Amabile N, Guérin AP, Leroyer A, Mallat Z, Nguyen C, Boddaert J, et al. Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. J Am Soc Nephrol. 2005;16:3381–8.
19. Paudel KR, Panth N, Kim D-W. Circulating endothelial microparticles: a key hallmark of atherosclerosis progression. Scientifica (Cairo). 2016;2016:1–9.
20. Helbing T, Olivier C, Bode C, Moser M, Diehl P. Role of microparticles in endothelial dysfunction and arterial hypertension. World J Cardiol. 2014;6:1135–9.