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Changes of Reactive Hyperemia Index (RHI) in Hypertensive Patients

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Abstract: Recent epidemiological evidences indicates a complex association of hypertension with vascular endothelial dysfunction which causes the development and progression of atherosclerosis leading to adverse cardiovascular and cerebrovascular events due to reduction in nitric oxide (NO) bioavailability. The aim of the study was to investigate the value on reactive hyperemia peripheral arterial tonometry (RH-PAT) as a noninvasive tool to assess and compare endothelial function in between hypertensive and normotensives. Although they do not measure vascular function in the coronary circulation directly, they have been shown to correlate reasonably with its more invasive counterparts. A total of 73 hypertensive patients and 48 normotensive patients were consecutively recruited from Cardiology outpatient department of first affiliated hospital of Fujian medical university. Peripheral endothelial function was measured by using EndoPAT device to assess reactive hyperemia induced vasodilation and expressed by the reactive hyperemia index (RHI) with age, sex, BMI, height with the control subjects and further the relationship between RHI and clinical characteristics laboratory cardiovascular risk factors were also investigated. Statistical analysis were performed using SPSS software version 19.0. The normality of the distribution of variables were performed by the Kolmogorov-Smirnov test and homogeneity test of variance. Continuous variables were expressed as mean ± SD and categorical variables were expressed as percentages. Comparison between two normal groups were made by t-test, for abnormal distributed variables Mann-Whitney U test was used and χ^2 test was performed for comparison of categorical variables. Pearson's correlation analysis was used to assess associations between measured parameters and a p-value < 0.05 was considered to be statistically significant. RHI was significantly lower in hypertensive subjects compared to normotensive subjects (1.69 ± 0.46 vs 2.27 ± 0.60, p < 0.001) and when both groups were divided into different subgroups according to (sex, age < or ≥ 55, smoking, high cholesterol, physical activity, BMI, alcohol habit), significantly low RHI was noted in the hypertensive subgroups, p < 0.05. In the both groups there was no significant difference in normal cholesterol category where as in hypertensive group, subjects with regular physical activity had significantly high RHI as compared to those with no physical activity at all. In a univariate analysis age (r = -0.482, P = 0.001), Systolic BP (r = -0.312, P = 0.001), GFR (r = 0.196, P = 0.031), physical activity (r = 0.536, P = 0.001) were found to significantly correlate with RHI. In a multivariate analysis, only age (β = 0.004, P = 0.001) and SBP (β = 0.003, P = 0.010) significantly and independently correlated with RHI. RHI was significantly attenuated in hypertensive subjects and showed significant correlation between age, SBP, GFR, and physical activity indicating endothelial dysfunction, suggesting RH-PAT may be used as a non-invasive test to identify hypertensive patients with an early endothelial dysfunction.

Keywords: Endothelial Dysfunction, Hypertension, Atherosclerosis, Cardiovascular Risk, Nitric Oxide, Oxidative Stress

1. Introduction

Hypertension is a long term medical condition in which blood pressure is persistently elevated in the arteries, classified as primary or secondary and is defined as chronic elevation of SBP ≥ 140mmHg and DBP ≥ 90mmHg [1] and is usually asymptomatic. 95% of all diagnosed cases of hypertension, categorized as primary hypertension lacks an identifiable trigger of B. P and remaining 5% categorized as secondary which are caused by kidney disease or tumor [2]. Hypertension is the most significant risk factor for cardiovascular disease, stroke heart failure, peripheral arterial disease, chronic kidney disease, vision loss [3] and one third

of premature deaths in men and one quarter of premature death in women are due to cardiovascular diseases [4]. Hypertension is characterized by vascular remodeling and increased increase in peripheral vascular resistance to blood flow [5] and may lead to the complication of hypertension [6]. The vascular endothelial function is impaired in hypertension [7-8]. The endothelium is a delicate monolayer of cells lining vascular inner layer and release variety of vasoactive substances including different vasodilators like nitric oxide (NO), Prostacyclin (PGI_2), bradykinin, and vasoconstrictors such as endothelin-1, thromboxane, angiotensin II, prostaglandin H2 (PGH2), and reactive oxygen species (ROS) and maintain vessel tone and growth, local regulation of hemostasis and coagulation, fibrinolysis, inflammation and immunological reaction and the properly functioning endothelium is the key to cardiovascular health. Endothelium constitutes a physical barrier between blood and tissues, alterations of one or more such actions may cause vascular endothelial dysfunction. In the hypertensive patients reduction of NO bioavailability causes endothelial dysfunction which is the early stage of atherosclerosis [9-11] which predisposes to thrombosis, leukocyte adhesion and smooth muscles cell proliferation [12-13] and increases the risk of cardiovascular and cerebrovascular diseases [14-17].

Furthermore, the evaluation of endothelial dysfunction can be useful for treating apparently healthy individuals who are at risk of developing cardiovascular diseases [18-19] to prevent major unfavorable cardiovascular morbidity and mortality [20-21]. Therefore, in order to promote testing of endothelial function for individuals at increased risk, at such level of disease that cannot be identified with classical imaging techniques, non-invasive assessment of vascular endothelial function is a mean to identify patients at increased risk of developing cardiovascular diseases [22]. Early detection of endothelial dysfunction have therapeutic and prognostic implications [23]. Recently, several non-invasive methods for assessing the endothelial dysfunction has been developed. Flow mediated dilation (FMD) is one such particularly useful technique, where the endothelial function is measured via ultrasound measurement of the brachial artery diameter during reactive hyperemia mediated by nitric oxide (NO) dependent vasodilatation [24-25]. However, the results of FMD can vary due to technical problems encountered during measurements, and it is operator dependent and expensive, so FMD is not standardized among institutions [26]. The gold standard for measuring endothelial function is coronary angiography, however due to complex nature of the procedure it has never been used outside research [27].

Endo-PAT 2000, FDA approved device was developed to overcome this problem, and to investigate the relationship between coronary and peripheral microvascular endothelial function, it is another noninvasive and rapid test for measuring changes in digital pulse volume during reactive hyperemia [28-29]. It has shown an 80% sensitivity and 86% specificity to diagnose coronary artery disease when compared against coronary angiography [33]. In PAT, the pulse wave amplitude is assessed before and during reactive hyperemia, which is induced by occluding blood flow of the brachial artery using an inflatable cuff. The calculated index (reactive hyperemia index; RHI) between the flow in the arm with reactive hyperemia and the control arm represents a measure of the endothelial function [30-31]. Studies using the Endo-PAT 2000 have shown that RHI score reflects NO bioavailability [32] and RHI correlates with the measurement of endothelial vasodilator function in the coronary arteries [33]. Patients with lower RHI score < 1.69 are associated with impaired endothelial function [34] and are risk of cardiovascular diseases [35-36] and lower RHI (indicating endothelial dysfunction) can be reversed with treatment [37].

The main aim of our study was to compare the peripheral endothelial function of hypertensive in compared with normotensives control groups.

2. Materials and Methods

2.1. Study Population

A total of 121 male and female patients aged (28 to 88) years old who visited the Cardiology outpatient department of the first affiliated hospital of Fujian Medical university were invited to participate in the present cross sectional study between October 2015 to May 2016. All of the recruited patients provided their informed consent and the approval was obtained from the Ethical Review Board of the first affiliated Hospital of Fujian Medical University.

The study populations were divided into 2 groups; 1) Hypertensive groups (n = 73, Male = 40) with the history of hypertension and with or without cardiovascular risk factors, and 2) Normotensive groups (n = 48, Male = 28) with no history of hypertension and with or without cardiovascular risk factors. Both groups were matched according to their age, height, BMI, BSA, cardiovascular risk factors (smoking, hyperlipidemia, arterial hypertension, positive family history of hypertension and diabetes, alcohol habits. Their physical activity was also documented and was grouped as no sports (NS) and physical activity ($\geq$ 4 times/ week).

We obtained the patients' demographic, medications and medical histories using a dedicated database, retrospectively. The patients' medical records were reviewed as described below. Arterial hypertension was defined as those taking anti-hypertensive medications or presented with an elevated systolic blood pressure of $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90mmHg after 15 minutes of rest, measured by mercury sphygmomanometer before endothelial function measurement according to the Chinese guideline of hypertension [38]. Diagnosis of essential hypertension was established on the basis of exclusion of secondary hypertension. Patients with Secondary hypertension including renal hypertension, primary hyperaldosteronism, Cushing's Syndrome, drug induced hypertension, obstructive sleep apnea, aortic valve disease, pregnancy was established and diagnosed by illness, history taking, physical examination, routine blood and urine test, and imaging.

Study participants with a plasma LDL Cholesterol > 2.5mmol/l, total cholesterol > 3.9mmol/l, triglyceride > 1.1mmol/l, or who were receiving cholesterol lowering therapy were classified as having hypercholesterolemia [39]. Smokers were defined as those who has smoked regularly during the previous 12 months [40] CKD was defined as an estimated glomerular filtration rate < 60ml/min / 1.73 m2. Those with severe hypertension (SBP ≥ 200mmHg and DBP ≥ 130mmHg), liver or renal disease, presence of any present or past history of cardiovascular or cerebrovascular diseases, diabetes mellitus, structural heart diseases, angina pectoris or heart failure (NYHA classes III–IV), hyperuricemia, homocysteinemia, malignant diseases, systemic inflammatory diseases, infectious diseases, taking alcohol > 60 ml/day were excluded from the study.

2.2. Laboratory Measurements

The complete blood cell counts total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, liver function test, fasting blood glucose, HBA1C, cysteine C, eGFR, BUN, creatinine, CRP, coagulation tests including fibrinogen was done after 12 hours of fasting via standard laboratory methods. An Echocardiography was also done to exclude any Structural or Valvular Heart Disease.

2.3. Assessment of Endothelial Function

The endothelial function was measured by PAT using an Endo-PAT 2000 device (Itamar Medical Ltd., Caesarea, Israel), which has been validated and used previously to assess the RHI as a measure of the microvascular endothelial function [41-42]. Endo-PAT was performed in all subjects after 8 hours of fasting during the (8: A. M to 12: P. M) and not having worked the night before. All the subjects were advised not to smoke, take alcohol or caffeine for at least 8 hour prior to the measurements. Reactive Hyperemic Index (RHI) was measured via Endo-PAT 2000 as described [37-38]. With the device, a beat to beat plethysmographic record of the finger arterial pulse wave amplitude can be captured with pneumatic probes. For this purpose, a pair of novel modified plethysmographic probes was placed on the index finger of each hand and the endothelium mediated peripheral arterial tone was recorded from both hand throughout the study. The reactive hyperemic technique was used to assess endothelial function and reactive and reactive hyperemia protocol consists of a 5 minutes baseline measurement, after which a blood pressure cuff on the right arm was inflated to 60mmHg above the baseline systolic blood pressure or at least 200 mmHg for 5 minutes. Occlusion of pulsatile arterial flow was confirmed by the reduction of the PAT tracing to zero. After 5 minutes, the cuff was deflated to induce reactive flow mediated hyperemia, which was measured for 5 minutes and the left hand remained un-occluded as a reference to correct for potential systemic changes. The ratio of the PAT signal after cuff release compared with the baseline was calculated through a computerized algorithm automatically normalizing for baseline signal and indexed to the contra-lateral arm. The calculated ratio reflects RHI.

2.4. Statistical Analysis

All data were fed into Excel to set up a data base. Statistical analysis was performed using SPSS software, version 19.0 (SPSS Inc., Chicago, USA). The normality of the distribution of the variables were firstly performed by the Kolmogorov–Smirnov test and homogeneity test of variances. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were expressed as percentages. Comparisons between two groups were made by analysis of t-test if the variables were normal distribution and equal variance. If the variables were abnormal distribution or not equal variance, comparisons among groups were made by the Mann-Whitney U test. The $\chi 2$ test was performed for comparison of categorical variables. Pearson's correlation analysis was used to assess associations between measured parameters. A p-value < 0.05 was considered to be statistically significant.

3. Results

3.1. Demographic, Clinical and Laboratory Characteristics of the Participants

The demographic, clinical and laboratory characteristics of the 2 groups are shown in Table 1. A total of 121 patients were studied, 48 were normotensives and 73 were hypertensives. There was statistically significant difference between groups with respect to age (p < 0.001), SBP (p < 0.001), GFR (p = 0.04), RHI (p < 0.001) and no difference was observed in variables like BMI, weight, height, heart rate, smoking%, alcoholic%, total cholesterol, HDL, LDL, glucose, HBA1C, creatinine.

Table 1. Demographic, Clinical and Laboratory Characteristics of the Participants.

variables	Normotensives (n = 48)	Hypertensives (n = 73)	P value
Age (years)	49.68 ± 11.71	60.87 = ± 12.05*	0.001
Gender (male %)	58.33%	54.79%	0.701
BMI(kg/m$^{2)}$)	24.22 ± 3.07	26 ± 4.07	0.13
BSA (m^2)	1.69 ± 0.19	1.73 ± 0.18	0.508
Weight(kg)	66.51 ± 11.95	69.58 ± 11.15	0.371
Height(cm)	165 ± 7.21	1.73 ± 0.18	0.491
Heart rate(bpm)	75.29 ± 12.05	74.05 ± 9.56	0.618
SBP (mmHg)	124.72 ± 10.91	145.19 ± 13.1*	0.001
DBP (mmHg)	81.31 ± 10.29	86.58 ± 13.61	0.139
Smoking %	25%	26%	0.899

variables	Normotensives (n = 48)	Hypertensives (n = 73)	P value
Alcoholic %	20%	26%	0.513
Positive family history of HTN %	54%	68.49%	0.111
Positive family history of DM %	21%	16.43%	0.541
Triglyceride (mmol/L)	1.1	1.33	0.069
Total cholesterol (mmol/L)	4.59 ± 1.29	4.76 ± 0.93	0.319
High density lipoprotein (mmol/l)	1.34 ± 0.33	1.31 ± 0.30	0.831
Low density lipoprotein (mmol/l)	2.92 ± 0.98	2.99 ± 1.05	0.804
Glucose (mmol)	5.4	5.7	0.79
HBA1C	5.5	5.5	0.742
Ejection fraction %	63.77 ± 4.30	69.69 ± 4.58	0.743
Creatinine (μmol/L)	64.86 ± 12.63	66.46 ± 12.46	0.901
eGFR (ml/min)	106.23 ± 11.58	97.58 ± 16.74*	0.041
RHI	2.27 ± 0.62	1.69 ± 0.46*	0.001

Abbreviation: BMI, body mass index; BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure; HTN, hypertension; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; RHI, reactive hyperemia index; *P < 0.05.

3.2. Comparison of RHI Between the 2 Groups

Average RHI was significantly higher in normotensives groups than the hypertensives (2.27 ± 0.60 vs 1.69 ± 0.46, P < 0.001), Figure1. When sub-dividing the 2 groups into (age, sex, smoking, alcohol, cholesterol, physical, BMI) categories, we found that normotensive subjects with (age < 55 or ≥ 55 years, males and females, smokers and non-smokers, alcoholics and non-alcoholics, high cholesterols, no physical activities and physical activities, high and normal BMI) had significantly higher RHI as compared to hypertensive subjects, as shown in the Table 2. There was no significant difference in the RHI in between the normotensive and hypertensives subjects in the normal cholesterol category (2.17 ± 0.61 vs 1.74 ± 0.48, p = 0.151). In the hypertensives subjects with physical activity (> 4days/week) had significant higher RHI than those with no physical activity in the same group (1.93 ± 0.47 vs 1.39 ± 0.21, p > 0.05), shown in the figure 2.
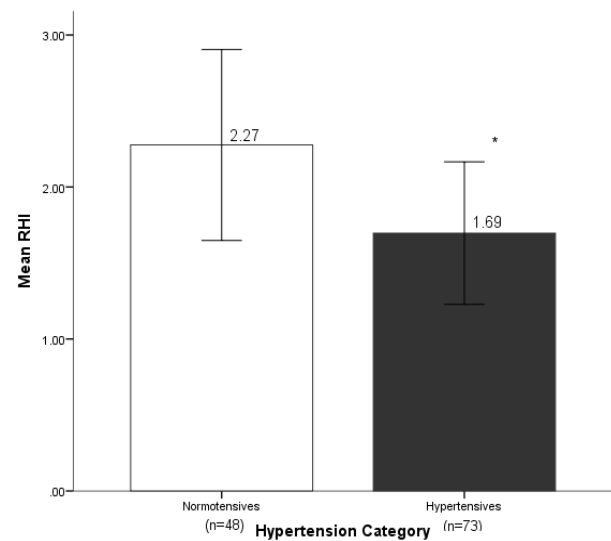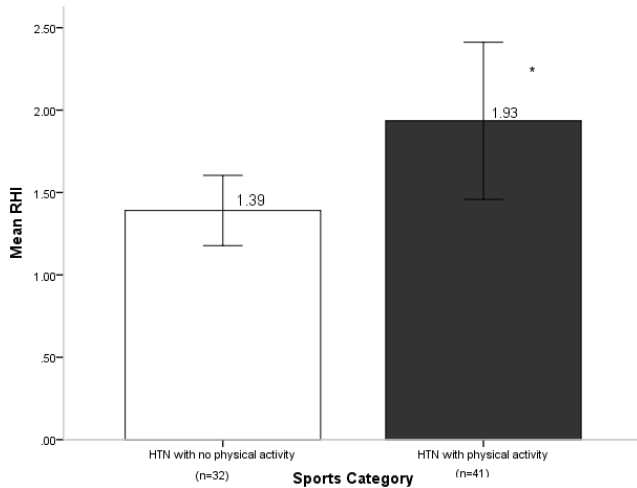

Figure 1. *Comparison of RHI between hypertensives and normotensives groups. *p < 0.05.*

Table 2. *Comparison of RHI between hypertensive and normotensive sub-groups.*

Variables Categories	RHI value Normotensive	RHI value Hypertensives	P value
Sex			
Males	(n = 28) 2.19 ± 0.40	(n = 40) 1.73 ± 0.45*	0.001
Females	(n = 20) 2.39 ± 0.84	(n = 33) 1.65 ± 0.48*	0.001
Age			
< 55years	(n = 35) 2.37 ± 0.65	(n = 20) 1.81 ± 0.56*	0.05
≥ 55 years	(n = 13) 2.00 ± 0.48	(n = 58) 1.65 ± 0.48*	0.003
Smoking			
non-smokers	(n = 36) 2.29 ± 0.69	(n = 54) 1.67 ± 0.44*	0.001
smokers	(n = 12) 2.22 ± 0.30	(n = 9) 1.75 ± 0.52*	0.001
Cholesterol			
normal cholesterol	(n = 18) 2.17 ± 0.61	(n = 14) 1.74 ± 0.48	0.151
high cholesterol	(n = 30) 2.33 ± 0.63	(n = 59) 1.75 ± 0.52*	0.001
Physical activity			
no physical activity	(n = 7) 1.76 ± 0.28	(n = 32) 1.39 ± 0.21*	0.001
physical activity	(n = 41) 1.93 ± 0.47	(n = 41) 1.93 ± 0.47*	0.001
BMI			
normal BMI	(n = 30) 2.26 ± 0.71	(n = 33) 1.65 ± 0.48*	0.001
high BMI	(n = 18) 2.29 ± 0.47	(n = 40) 1.67 ± 0.46*	0.001
Alcohol			
non alcoholic	(n = 38) 2.27 ± 0.68	(n = 54) 1.67 ± 0.44*	0.001
alcoholic	(n = 10) 2.28 ± 0.37	(n = 19) 1.75 ± 0.52*	0.004

Abbreviation: BMI, body mass index; *P < 0.05.

Figure 2. *Comparison of RHI in hypertensives sub-group with physical activity and no physical activity; *p < 0.05.*

3.3. Correlation and Regression Analysis Between RHI and Other Parameters

The correlation between RHI and each of the parameters are shown in Table 3. None of the evaluated parameters, except for age, SBP, GFR and physical activity showed any significant correlation with RHI in the Univariate Analysis. We next evaluated the Multivariate Correlation between RHI and these parameters in Table 4. A multiple regression analysis revealed that the age (β = 0.004, P = 0.0010 and Systolic blood pressure (β = 0.003, P = 0.010) significantly correlated with RHI and are an independent and significant variables associated with associated with endothelial function as measured by RH-PAT.

Table 3. *Univariate analysis of the correlation between endothelial function and various parameters.*

VARIABLE	r	P value
Age	-0.482	0.001
SBP	-0.312	0.001
eGFR	0.196	0.031
Sports activity	0.536	0.001

Abbreviation: SBP, systolic blood pressure; eGFR, estimated glomerular filtration.

Table 4. *Multivariate analysis for the association of RHI with various parameters.*

Variables	B	β	P value
Age	-0.021	0.004	0.001
SBP	-0.008	0.003	0.01

Abbreviation: SBP, systolic blood pressure.

4. Discussion

Our study demonstrates that the subjects with hypertension have a lower RHI as measured by RH-PAT compared to than those with normotensive subjects, suggesting hypertension as an independent risk factor for endothelial dysfunction. Our present study could represent hypertension as a greater risk

factor cardiovascular diseases as described in several epidemiological studies [43-45]. Cardiovascular risk factors such as smoking, [46-50] diabetes, [51-54] hypercholesterolemia, [55-56] advance age, [57-58] obesity, [59] physical activity, [60-62] can have an influence on endothelial function, we matched and sub-divided hypertensives and normotensives subjects according to these cardiovascular risk factors. In our present cross-sectional study we found age and systolic blood pressure had a negative and GFR and physical activity has a positive correlation with RHI in a univariate correlation analysis whereas systolic blood pressure and age are independently and significantly associated with RHI. Recent studies have shown the mechanism behind hypertension induced oxidative stress, increase in production of oxygen-derived free radicals [63], decreased level of anti-oxidant [64] and vascular inflammation in the pathogenesis of endothelial dysfunction leading to impairment of endothelium dependent vasodilation [65-66]. Number of studies on endothelial dysfunction shows the mechanism of decreased bioavailability of nitric oxide (NO) in hypertension either due to a decreased production or an increased degradation. Another study, has reported that increased amount of endogenous eNOS inhibitor, asymmetric dimethyl arginine, increased amount of vasoconstrictors like angiotensin II, endothelin 1 norepinephrine and inactivation of nitric oxide (NO) by reactive oxygen species (ROS) [67-68]. Taddei et al [69] has presented a result showing young offspring of essential hypertension patients having familial history of hypertension had a decreased response to acetylcholine linked to a defect in NO pathway suggesting endothelial dysfunction as a consequence of hypertension. Another evidence has suggested the degree of impairment of endothelial function related to the extent of blood pressure elevation, also suggesting endothelial dysfunction as a consequence of hypertension [70-71].

Importantly, our studies supports the other studies, in elderly and in animal models, the decline of endothelial dependent vasodilation function with aging [72-73]. Several studies have shown the decrease eNOS activity and nitric oxide (NO) activity in senescent endothelial cells [74-75]. Impairment of endothelial function with increasing age is mediated through accelerated NO degradation [76] reduced eNOS expression/action [77], increased production of ROS [78], inhibition of NOS activity by endogenous NOS inhibitors [79], inflammatory reactions and increased phosphodiesterase activity [80] and decreased endothelial progenitor cells [81].

Our results support the hypothesis that physical activity might cause an improvement in endothelial function [82-83] by exercise mediated increase in NO bioavailability and decrease in NO scavenging by ROS and reduction in oxidative stress. Adams et al [84] in a randomized controlled trial, demonstrated that exercise training for 4 weeks lead to a decrease in mRNA and protein expressions of the angiotensin II type receptor which is necessary for vasoconstriction effect of angiotensin II, leading improvement of endothelial

function.

Our study correlates with other studies about the relationship between GFR and cardiovascular morbidity and mortality [85]. Wim van et al [86] found that even a mild impairment of renal function (GFR < 90ml/min/1.73 m2) is a cardiovascular risk factor with a study of a ten year follow up of representative population based cohort comprised apparently healthy 8913 randomly selected participants. The KDIGO (Kidney Disease Improving Global Outcome) [87] and the NKF K-DOQI (National Kidney Foundation Dialysis Outcome Quality Initiative) [88] accepts an increased risk for secondary complication of CKD on CVD for a GFR of (60ml/min/1.7 m2). Our study is compatible with the study that the risk of CVD increases with accumulation of uremic substances with declining renal function [89] and CVD and renal failure are reciprocal, endothelial dysfunction might cause renal impairment and vice-versa [90]. The potential mechanisms for the impairment of endothelial function in the hypertensives subjects could not be elucidated from our data because that was not the aim of our study. According to our study design, the classic cardiovascular risk factors could have influenced our results.

5. Study Limitations

Current study has some limitations. Firstly, we have excluded diabetic patients due to small number of sample size and also the study was not designed to assess the difference in endothelial function in diabetic subjects. Secondly, enrolled subjects were who visited in our hypertension clinic to check up their cardiovascular health and our study had a small sample size, which might affect the statistical significance. Hence, the generalization of our results should be taken with careful attention and selection bias could exist. Indeed, no significant correlation between the endothelial dysfunction and cardiovascular risk factors like smoking, [39-43] hypercholesterolemia, [48-49] obesity [52] was noted, which showed significant association with endothelial dysfunction in other studies. Finally, we used RH-PAT 114 technique instead of FMD to assess endothelial function. Even though RH-PAT method has excellent reproducibility [91] for the measurement of endothelial function, no single method is proven to be superior. Also, single measurements of variables including hypertension and other parameters may not represent the exact measurements of subject conditions and we didn't evaluate the clinical outcomes of study subjects. Thus, further research should be directed at determining whether measurements of endothelial function can be used to guide treatment, change outcomes, and to ascertain whether detection of endothelial function will be useful in clinical area.

6. Conclusion

The present study demonstrates a significant reduction of RHI in hypertensive subjects, as measured my RH-PAT technique suggesting endothelial dysfunction. RHI showed a significant correlation with age, systolic blood pressure, GFR and physical activity. Also, higher RHI was noted in hypertensive subjects with physical activity than those with no physical activity suggesting exercise mediated improvement of endothelial function. Therefore, careful attention to the control of blood pressure and other cardiovascular risk factors causing disease and suggesting regular participation in physical activity is mandatory for the improvement of endothelial function to prevent future cardiovascular morbidity and mortality.

Acknowledgements

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