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Original article

Premature vascular deterioration in young patients affected by Wilson's disease: a pilot study

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Abstract

Introduction: Wilson's disease (WD) is a genetically inherited pathology which leads to an excessive deposition of copper in the human tissues, most of all in those of liver and brain. Even the cardiovascular system may be involved, although heart and vessels in those suffering from WD were fleetingly studied. This research aimed at evaluating the autonomic control of blood pressure (BP) and the endothelial function in a sample of young WD subjects.

Methods: Eleven WD individuals were recruited in the study (54% females; mean age and age at diagnosis: 16.3 ± 5.0 and 8.3 ± 4.0 years, respectively). BP was measured at the right arm (supine and upright after 3 minutes of standing) and ankle-brachial index (ABI) was evaluated as well. WD findings were compared with those of healthy peers (controls).

Results: In those with WD, systolic BP in the upright position raised when compared to the value in the supine position (128 ± 2 vs 112 ± 3 mmHg, $p < 0.002$), while declined in the controls. ABI was significantly lower in WD group (0.9 ± 0.2 vs 1.1 ± 0.1 in the control group, $p < 0.05$) and an inverse correlation was found between the disease duration and ABI as well ($r = -0.66$, $p < 0.03$).

Conclusions: These preliminary results suggest an early vascular deterioration in WD patients, notwithstanding their very young age and concomitant copper-chelating treatment. Although the heart and vessels are not the main target of WD, the detection of this unique population, potentially predisposed to cardiovascular accidents, suggests to enhance strategies of primary prevention.

Keywords

Wilson's disease, copper, dysautonomia, blood pressure, endothelium, prevention.

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Introduction

The Wilson's disease (WD) is a rare autosomal recessive disorder whose genetic defect lies in a mutation of the *ATP7B* gene, located on chromosome 13, which encodes an ATPase responsible for the copper transmembrane transport [1]. The result is the reduced hepato-biliary excretion of this metal, with accumulation in tissues, particularly in the liver [2]. WD was described for the first time by Kinnear Wilson in 1912 as a hepato-lenticular degeneration [3]. Its incidence in the world is 1:30,000 inhabitants, but in Sardinia it is significantly more frequent (approximately 1:3,000) [4-6]. The disease may manifest with hepatic involvement already in childhood or later (third decade of life), generally with neuropsychiatric symptoms [1, 7]. The diagnosis is usually made by determination of ceruloplasmin, the transport protein of copper in the blood, urinary excretion of copper in the 24 hours, evaluation of the accumulation of the metal in the liver, *via* biopsy, genetic analysis of the defect and, in adults, also by identifying the presence of the ocular ring of Kayser-Fleischer [7]. The therapy consists of copper-chelating drugs and of zinc [1, 8]. It is important to diagnose the disease early because, if not recognized and treated in time, WD may lead to a fatal outcome [1, 9].

The WD may cause events also at the level of cardiovascular system. Among these, the following have been described: *i.* morphological alterations of the ECG, until the appearance of arrhythmias; *ii.* dysautonomia, which manifests itself as orthostatic hypotension, or an abnormal response to the Valsalva maneuver (excessive lowering of blood pressure [BP] and/or appearance of marked bradicardia); *iii.* dilated cardiomyopathy, with heart failure and ventricular arrhythmias [10, 11]. In addition, the autopsy of patients who died of WD showed myocardial hypertrophy, disease of the small coronary vessels and focal inflammation [11].

Despite these sporadic reports, cardiovascular involvement during WD was, on the whole, quite neglected. Probably this was due to its late appearance and less clinical evidence, in comparison with other organs and systems. The purpose of this study was to evaluate the autonomic pressor response and the endothelial function in a group of young patients with WD. The results obtained were compared with those of a matched population of healthy control subjects.

Materials and methods

Population in the study

Eleven young patients with WD (5 males and 6 females; mean [± SD] age, 16.3 ± 5.0 years; weight, 66.7 ± 3.1 kg) were enrolled in the study at the 1st Department of Pediatrics, University Hospital of Cagliari (Italy). Six of them were still in the pediatric age, according to the definition used in the European Union (< 18 years) [12]. The diagnosis of WD in our patients was specified at the age of 8.3 ± 4.0 years, on the basis of: occasional finding of hypertransaminasemia, cupremia, cupruria and ceruloplasminemia, intrahepatic copper dosage after biopsy, and genetic analysis. All patients were receiving copper-chelating therapy (penicillamine, mean dose 600 ± 100 mg/24 hours), but one took trientine (900 mg/24 hours). Ten patients were also taking B6 vitamin. The control of the disease was monitored through the blood and urine copper concentrations, which, at the time of the study, amounted to 23.7 ± 22.1 mcg/dl and 449.8 ± 243.1 mcg/24 hours, respectively. Exclusion criteria were: presence of diabetes, dyslipidemia, arterial hypertension and other conditions influencing endothelial function, such as vasoactive drugs consumption and smoking. A group of 11 healthy subjects (6 males and 5 females; age, 16.6 ± 4.0 years; weight, 67.3 ± 4.2 kg) recruited among those visited for eligibility in non-competitive sports, comparable to the patients by age and physical characteristics, were used as controls. In these controls WD was excluded on the basis of urine and serum copper concentration measurement. All subjects, or their parents if under age, gave their written consent to the study, which was conducted in accordance with the revised Declaration of Helsinki.

The anthropometric, clinical and laboratory characteristics of all the examined WD subjects are summarized in **Tab. 1**.

All patients and controls were evaluated at the Operative Unit of Cardiology and Angiology of the same University. All of them underwent clinical examination including measurement of BP in the right arm (supine and upright) and in the ipsilateral ankle. ECG and transthoracic echocardiography, associated with tissue Doppler evaluation (TDI) were recorded.

Instrumental investigations. ECG was recorded using a standard 12-lead electrocardiograph (AR 1200 ADV, Cardiette® Cardioline®, Et Medical Devices S.p.A., Milan, Italy). ECG tracings were examined for the analysis of heart rate, wave morphology, duration and axis of QRS and other intervals (PR, QRS, QT, QT corrected for heart rate, and QT dispersion).

BP was measured in the right arm with a mercury sphygmomanometer (F. Bosch Medizintechnik, GmbH & Co. KG, Bisingen, Germany) and cuff of appropriate size, in a quiet environment with the subject in the supine position after not less than 15 minutes of acclimatization. The measurement was repeated in upright position after 3 minutes of standing. The systolic and diastolic BP were defined on the basis of the 1st and 5th Korotkoff sounds, respectively. For statistical analysis we used the average of three consecutive BP measurements, according with a protocol previously approved even in pediatric patients [13].

The ankle-brachial index (ABI) was calculated as the ratio between the systolic pressure measured at the level of the ankle (posterior tibial artery) and that in correspondence of the upper limb (brachial artery). ABI was measured with all the patients in the supine position, that is with the legs being horizontal. The same previously mentioned mercury sphygmomanometer was used. It was considered abnormal an ABI value < 0.9 [14].

Echocardiographic images were recorded using a commercially available system equipped with TDI (Toshiba APLIO CV ultrasound system-SSA 770A/CV, Toshiba Corp., Tochigi, Japan). Left ventricular ejection fraction was obtained from the apical 4- and 2-chamber views according to Simpson's rule and was considered abnormal under 55%. Pulsed wave Doppler examination of the left ventricle inflow from the 4-chamber view was performed with the sample volume placed between the mitral leaflet tips and early (E) and late (A) diastolic peak velocities were calculated; E deceleration time (DecT) was measured and E/A ratio subsequently derived. Longitudinal function was evaluated using pulsed TDI at the mitral

Table 1. Characteristics of the patients suffering from Wilson's disease (WD).

	Gender	Genetic diagnosis	Age at diagnosis (years)	Age at study (years)	Age from diagnosis (years)	Therapy	Blood copper concentration (mcg/dL)	Urine copper concentration (mcg/24h)
1	Female	-441/-427del/V1146M	6	21	15	penicillamine (750 mg/24h)	6	290
2	Male	-441/-427del/V1146M	5	25	20	penicillamine (750 mg/24h)	16	931
3	Male	-441/-427del/V1146M	1	20	19	penicillamine (600 mg/24h)	13	780
4	Male	2304-2305insC/ H1069Q	9	14	5	penicillamine (600 mg/24h)	34	445
5	Female	-441/-427del/H1069Q	6	21	15	penicillamine (750 mg/24h)	38	328
6	Male	-441/-427del omo	7	11	4	penicillamine (450 mg/24h)	9	510
7	Female	-441/-427del omo	15	19	4	trientine (900 mg/24h)	24	135
8	Female	-441/-427del omo	12	16	4	penicillamine (600 mg/24h)	83	124
9	Male	-441/-427del omo	5	8	3	penicillamine (600 mg/24h)	11	476
10	Female	-441/-427del/N1270S	13	19	6	penicillamine (600 mg/24h)	12	468
11	Female	-441/-427del/N1270S	9	14	5	penicillamine (600 mg/24h)	15	461

annulus, placing the sample volume in the basal segment of the interventricular septum from the apical 4-chamber view: peak velocities in systole (Sm), isovolumic relaxation time (IVRT), early (Em) and late (Am) diastole were measured. For more accurate measurements, TDI curves were obtained from raw data analysis. All examinations were performed by the same experienced cardiologist. A simultaneous electrocardiographic tracing was also obtained. Reproducibility of TDI parameters in our laboratory had been previously demonstrated [15].

Statistical analysis

The results of WD patients were compared with those of controls using the non-parametric Student t test for samples distributed in non-Gaussian way. The continuous variables were analyzed with the chi-square test. The relationships between the various parameters were studied with univariate analysis, calculating the Pearson correlation coefficients and plotting the corresponding regression lines. Multivariate analysis was not applied, because of the low sample size. The minimum level of statistical significance was established for values of $p < 0.05$. All statistical analyses were performed using a commercial software (SPSS® version 19.0, SPSS Inc., Chicago, Illinois, USA).

Results

There were no appreciable differences in ECG, standard and TDI echocardiographic examinations performed at baseline between WD subjects and controls.

Systolic BP in the upright position, measured after 3 minutes of standing as previously mentioned, increased in comparison to the value in the supine position in WD patients (128 ± 2 vs 112 ± 3 mmHg, $p < 0.002$), while decreased in the control group. An increase equal to or greater than 10 millimeters of mercury in systolic BP after assuming the standing position is considered as orthostatic hypertension. Moreover, an increase in diastolic BP after 3 minutes of standing position, expression of a physiological vasoconstriction, was observed both in patients (p = ns) and controls.

The value of ABI appeared significantly lower in WD patients, due to a pressure measured at the ankle less than that detected in the ipsilateral arm (0.9 ± 0.2 vs 1.1 ± 0.1 in controls, $p < 0.05$; see also **Tab. 2** and **Tab. 3**). A significant inverse relationship was found between WD duration and the value of the ABI ($r = -0.66$, $p < 0.03$; **Fig. 1**).

Discussion

A mutual interaction between the function of the heart and the liver and a large number of acute and chronic diseases that affect both these two organs were described [16].

Our findings document an early cardiovascular dysfunction in patients of both sexes, adolescents or young adults with WD. Such alterations consist of *i.* an erroneous postural adaptation of BP and *ii.* an impaired vasomotor response, documented by the inversion of the ABI.

The first sporadic reports of an involvement of the cardiovascular system in patients with WD concerned electrocardiographic abnormalities (such as left and bi-ventricular hypertrophy, repolarization abnormalities, extrasystolic beats, atrial abnormalities at basal ECG with consequent onset of atrial fibrillation, and conduction disturbances) and a moderate prevalence (19%) of orthostatic hypotension [10, 17]. In addition, it was reported a significant prevalence (33%) of an abnormal response to the Valsalva maneuver, with significant lowering of BP and/or occurrence of severe bradycardia [10]. Two patients died during the study, respectively for the onset of ventricular fibrillation and for severe heart failure secondary to dilated cardiomyopathy. The autopsy revealed a myocardial content of copper of 2.28 mcg/g [10].

Table 2. Blood pressure (BP) values in Wilson's disease (WD) patients and controls.

	Supine	Orthostatism	p-value
WD systolic BP	112 ± 3	128 ± 2	< 0.002
WD diastolic BP	78 ± 2	80 ± 2	ns
C systolic BP	110 ± 1	105 ± 4	< 0.05
C diastolic BP	77 ± 2	83 ± 3	< 0.05

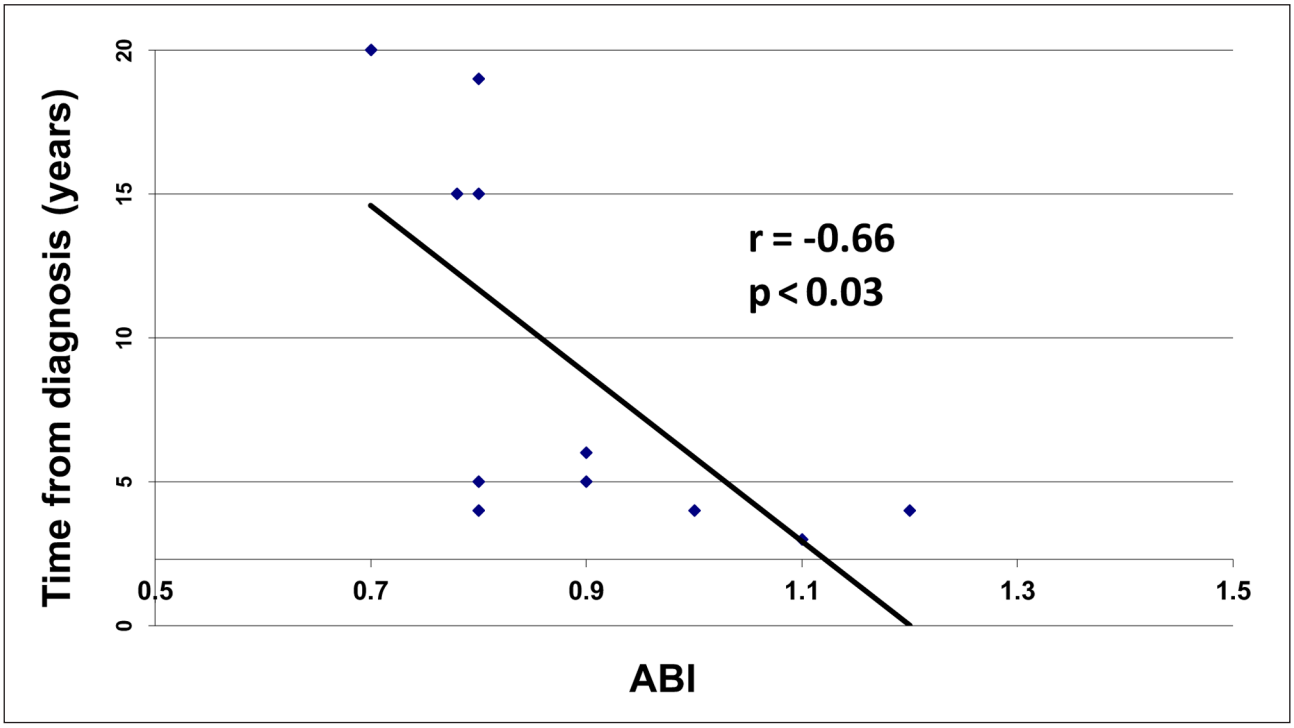
WD: Wilson's disease; C: controls; BP: blood pressure

Table 3. Blood pressure (BP) values in Wilson's disease (WD) patients and controls.

	Ankle	Brachial artery	ABI
WD systolic BP	112 ± 2	124 ± 2	0.9 ± 0.2
C systolic BP	110 ± 1	100 ± 1	1.1 ± 0.1

Statistical significance ABI in WD vs ABI in C: < 0.05.
ABI: ankle-brachial index; WD: Wilson's disease; C: controls; BP: blood pressure

Figure 1. Correlation between ankle-brachial index (ABI) and time spent from Wilson's disease (WD) diagnosis.



ABI: ankle-brachial index.

It is unclear whether chelation therapy may lead to an improvement in the electrocardiographic and BP abnormalities in these WD patients [18].

The abrupt rise in BP observed in our young patients on changing from the supine to the standing position confirms the existence of an autonomic dysfunction, previously reported among the cardiovascular manifestations of WD. Dysautonomia, often subclinical, is common in WD and is probably of central origin. Sympathetic and parasympathetic functions are equally affected [10, 19]. In the previous report, dating back to almost 3 decades ago, patients had a very variable age (9-47 years), on average higher than that of the present study [10]. In addition, about one third of patients did not undertake any copper-chelating therapy [8]. These dissimilarities could explain the different manifestation of dysautonomia in our patients, which manifested orthostatic hypertension instead of postural hypotension, as previously described. However, in another study it was reported that WD affects parasympathetic (with consequent BP lowering) rather than sympathetic functions (which usually leads to a rise in BP values) [20].

Although the precise mechanism of the inability to regulate BP in WD has not yet been elucidated in detail, it was attributed to a hyper-reactivity of alpha adrenergic receptors [21, 22]. However, it is a fact that the prevalence of orthostatic hypertension in the general population amounts to 1.1% of subjects, while among the WD patients we studied it affected no less than 73% of cases [23]. It is worth to mention that the systolic pressure increase in orthostatism is associated, in a later age, with an increased occurrence of peripheral arterial disease, silent cerebral ischemia, and stroke [21, 22, 24-26].

The ABI is a simple, inexpensive and repeatable test to measure in a non-invasive way the endothelial function [26]. A value lower than 0.9 is pathological. This cut-off has a sensitivity of 95% in young individuals and a specificity very close to 100% [13]. A low ABI indicates a slow propagation of the pressure wave between the upper and the lower body. Its reduction, suggestive of peripheral arterial dysfunction, is unanimously considered to be the signal of an increased cardiovascular risk, as well as pre-clinical atherosclerotic disease [27-29].

The ABI was reduced in the young WD patients we studied [30]. According to what we know, its impairment in the framework of WD should be interpreted as predictive of adverse cardiovascular events. About the mechanism of reduced ABI in our population, it can be hypothesized that endothelial accumulation of copper directly

inhibits acetylcholine and, in turn, reduces the bioavailability of nitric oxide. On the other hand, an identical mechanism was demonstrated in diabetic rabbits, in whose endothelium the copper was present to a greater extent than in healthy controls [31].

Regarding the lack of significant findings at echocardiographic examination in our WD sample, it may have been due to the relatively simple echocardiographic techniques that were used (Doppler, TDI), since at more sensitive strain and strain rate echo facilities some significant findings were reported [32].

The main limitation of this study is the small number of patients examined; it is, moreover, objectively difficult to recruit WD subjects in developmental age, because of the rarity of the disease. Secondly, we note that Guidelines, although recommend the accurate determination of the ABI for risk assessment, do not detail the method of execution and computing this index. Consequently, the large variety of calculation methods used could have, at least in part, influenced the different prevalence of peripheral arterial disease in our WD patients [33]. In this respect, determining ABI by means of an oscillometric BP device would be considered a more objective method. However, the ability of the selected cardiologist (P.P.B.) in measuring BP with a mercury sphygmomanometer was previously tested in a wide sample of about 1,000 pediatric patients. In addition, the cuff deflated slowly, that is with a ratio of 2 mmHg per second [13].

In conclusion, although the cardiovascular system is not the main target of WD, we demonstrated an early neuro-vascular involvement, despite the very young age of our patients and the concomitant copper-chelating therapy. The identification of this unique population, potentially vulnerable to adverse cardiovascular events, suggests the adoption of an appropriate strategy of primary prevention, possibly even personalized [34]. In order to continue and extend this study, we plan to measure the concentration of some markers of endothelial function (eg. nitric oxide inhibitors, such as endothelin and asymmetric dimethylarginine [ADMA]), and the use of plethysmographic techniques in the assessment of vasodilation reserve [35-38].

Declaration of interest

The Authors declare that there is no conflict of interest.

References

1. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. Lancet. 2007;369:397-408.
2. Luca P, Demelia L, Lecca S, Ambu R, Faa G. Massive hepatic hemosiderosis in Wilson's disease. Histopathology. 2000;37:187-9.
3. Wilson SAK. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain. 1912;34:295-509.
4. Loudianos G, Dessi V, Lovicu M, Angius A, Figus A, Lilliu F, De Virgiliis S, Nurchi AM, Deplano A, Moi P, Pirastu M, Cao A. Molecular characterization of Wilson disease in the Sardinian population – evidence of a founder effect. Hum Mutat. 1999;14: 294-303.
5. Zappu A, Magli O, Lepori MB, Dessì V, Diana S, Incollu S, Kanavakis E, Nicolaidou P, Manolaki N, Fretzayas A, De Virgiliis S, Cao A, Loudianos G. High incidence and allelic homogeneity of Wilson disease in 2 isolated populations: a prerequisite for efficient disease prevention programs. J Pediatr Gastroenterol Nutr. 2008;47:334-8.
6. Gialluisi A, Incollu S, Pippucci T, Lepori MB, Zappu A, Loudianos G, Romeo G. The homozygosity index (HI) approach reveals high allele frequency for Wilson disease in the Sardinian population. Eur J Hum Genet. 2013;21:1308-11.
7. Pilloni L, Lecca S, Coni P, Demelia L, Pilleri G, Spiga E, Faa G, Ambu R. Wilson's disease with late onset. Dig Liver Dis. 2000;32:180.
8. Roberts EA, Schilsky ML, American Association for Study of Liver Diseases (AASLD). Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008;47:2089-111.
9. Merle U, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. Gut. 2007;56;115-20.
10. Kuan P. Cardiac Wilson's disease. Chest. 1987;91:579-83.
11. Factor SM, Cho S, Sternlieb I, Scheinberg IH, Goldfisher S. The cardiomiopathy of Wilson's disease. Myocardial alteration in nine cases. Virchows Arch A Pathol Anat Histol. 1982;397:301-11.
12. van Esso D, del Torso S, Hadjipanayis A, Biver A, Jaeger-Roman E, Wettergren B, Nicholson A; Primary-Secondary Working Group (PSWG) of European Academy of Paediatrics (EAP). Paediatric primary care in Europe: variations between countries. Arch Dis Child. 2010;95:791-5.
13. Marras AR, Bassareo P, Ruscazio M. The prevalence of paediatric hypertension, emphasising the need to use specific population references: the Sardinian Hypertensive Adolescents Research Program study. Cardiol Young. 2009;19:233-8.
14. Xu D, Li J, Zou L, Xu Y, Hu D, Pagoto SL, Ma Y. Sensitivity and specificity of the ankle – brachial index to diagnose peripheral artery disease: a structured review. Vasc Med. 2010;15:361-9.
15. Bassareo PP, Tumbarello R, Piras A, Mercuro G. Evaluation of regional myocardial function by Doppler tissue imaging in univentricular heart after successful Fontan repair. Echocardiography. 2010;27:702-8.
16. Møller S, Bernardi M. Interactions of the heart and the liver. Eur Heart J. 2013;34:2804-11.

17. Arat N, Kacar S, Golbasi Z, Akdogan M, Sokmen Y, Kuran S, Idilman R. P wave dispersion is prolonged in patients with Wilson disease. World J Gastroenterol. 2008;14:1252-6.
18. GuiZhi L, Yan Z, Chao C. Ambulatory electrocardiogram changes with chelation therapy in 28 Wilson's disease patients. Life Sci J. 2014;11:1037-40.
19. Meenakshi-Sundaram S, Taly AB, Kamath V, Arunodaya GR, Rao S, Swamy HS. Autonomic dysfunction in Wilson's disease – a clinical and electrophysiological study. Clin Auton Res. 2002;12:185-9.
20. Soni D, Shukla G, Singh S, Goyal V, Behari M. Cardiovascular and sudomotor autonomic dysfunction in Wilson's disease – limited correlation with clinical severity. Auton Neurosci. 2009;151:154-8.
21. Fessel J, Robertson D. Orthostatic hypertension: when pressor reflexes overcompensate. Nat Clin Pract Nephrol. 2006;2:424-31.
22. Kario K, Eguchi K, Hoshide S, Hoshide Y, Umeda Y, Mitsuhashi T, Shimada K. U-curve relationship between orthostatic blood pressure change and silent cerebrovascular disease in elderly hypertensives: orthostatic hypertension as a new cardiovascular risk factor. J Am Coll Cardiol. 2002;40:133-41.
23. Wu JS, Yang YC, Lu FH, Wu CH, Chang CJ. Population-based study on the prevalence and correlates of orthostatic hypotension/hypertension and orthostatic dizziness. Hypertens Res. 2008;3:897-904.
24. Fan XH, Sun K, Zhou XL, Zhang HM, Wu HY, Hui RT. Association of orthostatic hypertension and hypotension with target organ damage in middle and old-aged hypertensive patients. Zhonghua Yi Xue Za Zhi. 2011;91:220-4.
25. Yatsuya H, Folsom AR, Alonso A, Gottesman RF, Rose KM; ARIC Study Investigators. Postural changes in blood pressure and incidence of ischemic stroke subtypes – The ARIC study. Hypertension. 2011;57:167-73.
26. Al-Qaisi M, Nott DM, King DH, Kaddoura S. Ankle brachial pressure index (ABPI): An update for practitioners. Vasc Health Risk Manag. 2009;5:833-41.
27. Grenon SM, Gagnon J, Hsiang Y. Video in clinical medicine. Ankle-brachial index for assessment of peripheral artery disease. N Engl J Med. 2009;361:e40.
28. Ikeda N, Araki T, Sugi K, Nakamura M, Deidda M, Molinari F, Meiburger KM, Acharya UR, Saba L, Bassareo PP, Di Martino M, Nagashima Y, Mercuro G, Nakano M, Nicolaides A, Suri JS. Ankle-brachial index and its link to automated carotid ultrasound measurement of intima-media thickness variability in 500 Japanese coronary artery disease patients. Curr Atheroscler Rep. 2014;16:393.
29. Wild SH, Byrne CD, Smith FB, Lee AJ, Fowkes FG. Low ankle-brachial pressure index predicts increased risk of cardiovascular disease independent of the metabolic syndrome and conventional cardiovascular risk factors in the Edinburgh Artery Study. Diabetes Care. 2006;29:637-42.
30. Fowkes FG,Murray GD, Butcher I, Heald CL, Lee RJ, Chambless LE, Folsom AR, Hirsch AT, Dramaix M, deBacker G, Wautrecht JC, Kornitzer M, Newman AB, Cushman M, Sutton-Tyrrell K, Lee AJ, Price JF, d'Agostino RB, Murabito JM, Norman PE, Jamrozik K, Curb JD, Masaki KH, Rodríguez BL, Dekker JM, Bouter LM, Heine RJ, Nijpels G, Stehouwer CD, Ferrucci L, McDermott MM, Stoffers HE, Hooi JD, Knottnerus JA, Ogren M, Hedblad B, Witteman JC, Breteler MM, Hunink MG, Hofman A, Criqui MH, Langer RD, Fronek A, Hiatt WR, Hamman R, Resnick HE, Guralnik J, McDermott MM. Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: a meta-analysis. JAMA. 2008;300:197-208.
31. Shukla N, Thompson CS, Angelini GD, Mikhailidis DP, Jeremy JY. Low micromolar concentrations of copper augment the impairment of endothelium-dependent relaxation of aortae from diabetic rabbits. Metabolism. 2004;53:1315-21.
32. Karakurt C, Çelik S, Selimoğlu A, Varol I, Karabiber H, Yoloğlu S. Strain and strain rate echocardiography in children with Wilson's disease. Cardiovasc J Afr. 2016;27:307-14.
33. Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, Hiratzka LF, Murphy WR, Olin JW, Puschett JB, Rosenfield KA, Sacks D, Stanley JC, Taylor LM Jr, White CJ, White J, White RA, Antman EM, Smith SC Jr, Adams CD, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Hunt SA, Jacobs AK, Nishimura R, Ornato JP, Page RL, Riegel B. ACC/AHA 2005 practice guidelines for the management of patients with peripheral arterial diseases (lower extremity, renal, mesenteric and abdominal aortic): a collaborative report from the American association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Interventional Radiology, Society for Vascular Medicine and Biology, and the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to develop guidelines for the management of patients with peripheral arterial disease). Circulation. 2005;113:e463-654.
34. Fanos V. 10 P Pediatrics: notes for the future. J Pediatr Neonat Individual Med. 2016;5(1):e050101.
35. Moerland M, Kales AJ, Schrier L, van Dongen MG, Bradnock D, Burggraaf J. Evaluation of the EndoPAT as a Tool to Assess Endothelial Function. Int J Vasc Med. 2012;2012:904141.
36. Bassareo PP, Fanos V, Puddu M, Flore G, Mercuro G. Advanced intrauterine growth restriction is associated with reduced excretion of asymmetric dimethylarginine. Early Hum Dev. 2014;90:173-6.
37. Bassareo PP, Puddu M, Flore G, Deidda M, Manconi E, Melis A, Fanos V, Mercuro G. Could ADMA levels in young adults born preterm predict an early endothelial dysfunction? Int J Cardiol. 2012;159:217-9.
38. Bassareo PP, Fanos V, Puddu M, Demuru P, Cadeddu F, Balzarini M, Mercuro G. Reduced brachial flow-mediated vasodilation in young adult ex extremely low birth weight preterm: a condition predictive of increased cardiovascular risk? J Matern Fetal Neonatal Med. 2010;23(S3):121-4.