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ORIGINAL ARTICLE

Biomarkers of endothelial dysfunction (vWF), hypofibrinolysis (PAI-1) and metabolic syndrome components in hypertensive patients with and without thrombotic complications

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Abstract

Background Essential hypertension is associated with increased risk for atherothrombotic disease.

Objective The aims were to determine and compared the plasma concentration of von Willebrand Factor (vWF) and Plasminogen Activator Inhibitor type 1 (PAI-1) in hypertensive patients with and without atherothrombotic disease and the influence of antihypertensive treatment on those biomarkers.

Methods Total of 341 individuals were included, 83 normotensive subjects and 258 hypertensive patients (43 with and 215 without atherothrombotic disease). The vWF and PAI-1 were measured by ELISA technique. Multivariate linear and quantile regression analysis were performed.

Results There was higher vWF concentration ($p < 0.001$) and PAI-1 ($p < 0.001$) in hypertensive compared with normotensive individuals. The vWF level was correlated with hypertension, older age and glucose, explaining 26%, 70% and 86% of vWF variability. Increased PAI-1 levels were correlated with glucose, triglycerides, HbA1c, explaining 66%, 73% and 90% of variability. In contrast, lower PAI-1 concentration was determined by older age.

Conclusions We found higher levels of vWF and PAI-1 in hypertensive patients, with highest concentration of vWF in patients with hypertension and thrombotic disease and the highest of PAI-1 in hypertensive patients without atherothrombotic disease. The lowest level of vWF was determined in patients with angiotensin II receptor blocker, and for the PAI-1 level in patients with calcium channel blocker medication. The lowest concentration of both biomarkers was present in patients who were treated with 3 or more drugs. Hypertension, older age, disorders in glucose and lipid metabolism were the main determinants of vWF and PAI-1 variability.

Highlights

1. We demonstrated increased levels of vWF and PAI-1 in hypertensive patients compared to normotensive individuals.
2. The highest level of vWF was found in hypertensive patients with atherothrombotic disease compared to patients with hypertension or normotensive individuals. The lowest concentration was registered in patients with angiotensin II receptor blocker monotherapy treatment. Meanwhile, the highest level of vWF was found with monotherapy of calcium channel blocker.
3. Moreover, the highest concentration of PAI-1 was determinated in patients with hypertension before atherothrombotic disease compared to hypertensive patients with atherothrombotic disease or controls. The lowest level of PAI-1 was observed in hypertensive patients with calcium channel blocker medication and it was similar to the level obtained from the control group.
4. The lowest concentration of vWF and PAI-1 was found in patients with 3 or more antyhypertensive drugs.
5. Hypertension, age and FPG determine up to 86% of variability of vWF plasma levels, while FPG, HbA1c, triglycerides and age determine up to 90% of the variability of PAI-1 plasma concentration in this group of hypertensive patients without T2 Diabetes Mellitus. Our results, demonstrated that in hypertensive patients the vWF concentration could be positive regulated by glucose levels, even without the presence of T2 diabetes mellitus.
6. The increased levels vWF are associated with endothelial damage/dysfunction and high levels of PAI-1 are associated with hypofibrinolytic activity. Therefore, we consider that hypertensive patients with uncontrol of blood pressure, as well as elevated vWF and PAI-1 concentration, body mass index, glucose and lipid parameters are in high risk to develop thrombosis complication such myocardial infarction and/or ischemic stroke.
7. Therefore, we propose that vWF and PAI-1 determination should be included as part of the monitoring and control in hypertensive patients and more relevant in those with a difficult blood pressure and metabolic components control which can generate endothelial damage and hypofibrinolytic state which are an important mechanism in atherothrombotic disease in this type of patients.
8. Also, we consider that combination of two or more antihypertensive drugs should be consider as a treatment for patients with hypertension, instead as a monotherapy and most important in patients with no good control of blood pressure.

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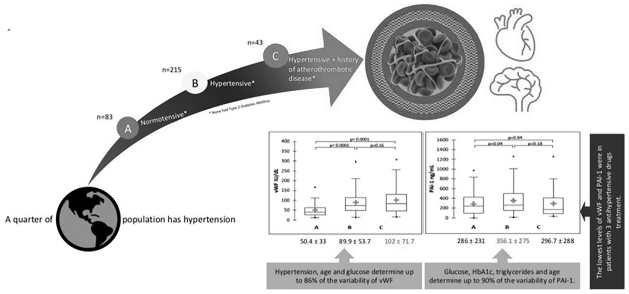
Graphical Abstract



Keywords Metabolic syndrome · Type 2 Diabetes Mellitus · Essential hypertension · Endothelial dysfunction · Hypofibrinolysis · Von Willebrand Factor · Plasminogen Activator Inhibitor type 1

Introduction

Essential hypertension is a major health problem, and it represents an important risk factor for atherothrombotic disease [1]. Approximately a quarter of the world's population has hypertension and only a third of those receiving pharmacological treatment for this disease have blood pressure controlled, and this lack of control could be associated with long time exposure of high vascular pressure [1].

Endothelial dysfunction and alterations in the fibrinolytic system are present in patients with hypertension, which is associated with myocardial infarction and stroke [2]. The vWF is a large, circulating glycoprotein that is synthesized by and stored in endothelial cells and platelets. The vWF mediates platelet adhesion to the vascular wall and platelet aggregation. The increased plasma concentration of vWF has been associated with endothelial cell damage/dysfunction, [3] which plays an important role in the hypertension development and is associated with coronary risk and adverse prognosis [4]. Previous studies had proposed that vWF plasma levels can be lowered by antihypertensive medication, [5] but results are still unclear.

Also, it has been documented the presence of hemostatic disturbance such impaired fibrinolysis in patients with hypertension. Plasminogen activator inhibitor type-1 (PAI-1) is the principal inhibitor of t-PA and u-PA, it is synthesized by endothelial cells, platelets, hepatocytes, smooth muscle and macrophages. PAI-1 is a physiologic inhibitor of fibrinolysis and it reduces clot lysis by preventing the t-PA from acting on its substrate plasminogen. The plasma concentrations of PAI-1, are influenced by age, gender, body mass index (BMI), circadian rhythm, obesity, levels of insulin and angiotensin [6]. Increased plasmatic levels of PAI-1 are present in patients with hypertension [7] and atherothrombotic disease [8]. There are some reports about the possible influence of the antihypertensive treatment on the PAI-1 plasma levels, but results are still controversial in different population worldwide [9].

The von Willebrand factor is a major marker for endothelial dysfunction and increased level of PA-1 is associated with a hypofibrinolytic state, and both mechanisms are relevant in the pathophysiology of hypertension, which represent a risk factor for atherothrombotic disease. Moreover, there are contradictory results about the possible effect of antihypertensive therapy on those biomarkers in patients with essential hypertension, probably due by genetic background. The aims of the present study were: 1) To determine and compared the plasma concentration of vWF and PAI-1 between normotensive individuals and hypertensive patients with and without atherothrombotic disease, 2) To identify the variables of study are associated with the variation of the plasmatic levels of vWF and PAI-1, and 3) To evaluate the influence of antihypertensive treatment on the vWF and PAI-1 plasma concentration.

Materials and methods

We performed a study in a secondary care level hospital at Mexico City from March 2018 to March 2019. We screened 258 consecutive patients with diagnosis of EH and ≥ 20 years old without Type 2 Diabetes Mellitus or dyslipidemia, who came for routine follow-up medical consultation. The reference group was formed by a total of 83 apparently healthy individuals (clinical and normal laboratory parameters), without history of hypertension, who were interested to know their cardiovascular risk. The recruitment was made by invitation through printed announcements and personal appeal to people to participate in the survey. We included all individuals who accepted to participate by informed written

consent. However, only 90% of the subjects who received the invitation accepted to participate. The study protocol was reviewed and approved by the Human Ethical Committee, and conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Demographic and clinical data such as age, sex, smoking, and history of dyslipidemia, diabetes mellitus, hypertension and atherothrombotic disease as well as pharmacological treatment were collected during an interview performed by a physician. After the interview anthropometric parameters such as body weight, height, BMI, and blood pressure (BP) were also taken. Also, biochemical parameters were measured 8 h fast and included: fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and triglycerides. The plasma concentration of vWF (Diagnostica Stago, Inc. NJ) and PAI-1 (Biopool International, Ventura, CA) were determined immunoenzymatically by enzyme-linked immunosorbent assay (ELISA).

Operational definitions

Diagnosis of EH was made when 1) it was referred during the interview, 2) subject had medication for hypertension, or 3) BP at the interview and one week later was ≥ 140 mmHg systolic BP (SBP) or ≥ 90 mmHg diastolic BP (DBP). A previous myocardial infarction or stroke, were considered has history of atherothrombotic disease. Myocardial infarction was based on history of electrocardiogram, clinical data and laboratory accordance with the European Society of Cardiology guidelines. Stroke was considered in all patients with history of focal neurological deficit with duration greater than 24 h followed by confirmation with brain-computed tomography or magnetic resonance; those with history of cardiac, carotid or vertebral artery sources of emboli were discharged. Dyslipidemia was considered if they had cholesterol ≥ 200 mg/dL, or triglycerides ≥ 150 mg/dL, or if they were being already treated. Smokers were considered if they were currently smoking or had ceased within the last 12 months. Patients with Type 2 Diabetes Mellitus (T2DM) or under hypoglycemic treatment were not included at the time they were enrolled.

Statistical analysis

Normality tests were carried out for quantitative variables using the Kolmogorov Smirnoff test. Numerical variables with normal distribution were expressed as mean ± standard deviations (SD), those with non-normal distribution were expressed as median and interquartile range (IQR). Categorical data were expressed in the number of observations (n) and percentages (%). Biomarkers of endothelial dysfunction (vWF), hypofibrinolysis (PAI-1), the metabolic syndrome components (BMI, FPG, HbA1c, total cholesterol, LDL-c, HDL-c, triglycerides) and age were compared between the reference group (normotensive subjects) and hypertensive patients with and without history of atherothrombotic disease. A one-way analysis of variance (ANOVA) or Kruskal Wallis test was used for comparison between groups for variables with normal and non-normal distribution, respectively. Categorical variables (gender, dyslipidemia, smoking) were compared using Chi square test. A Pearson correlation analysis was performed between continuous variables (age, BMI, SBP, DBP, FPG, HbA1c, total cholesterol, LDL-c, HDL-c, triglycerides) with vWF and PAI-1 plasma levels. A Spearman correlation analysis was performed between categorical variables (gender, dyslipidemia, and smoking status) with vWF and PAI-1 plasma levels. A multivariate linear regression (MLR) analysis was performed to estimate the independent contribution of each feature to the conditional mean variation in vWF and PAI-1 plasma levels, represented by a β-coefficient with a 95% confidence interval (95% CI). Only variables with statistical significance in the bivariate analysis were included in the model, a p value ≤ 0.05 (two-tailed) was considered statistically significant. Subsequently, a quantile regression model was performed to explore the relationship between independent variables and conditional quantiles of the dependent variable, by a two-step analysis, first we identify the quantiles for which the coefficients of the quantile regression are quite far from those of the analysis of covariance (ANCOVA), then we analyzed the selected quantiles to know the goodness of fit coefficients of the model for a specific quantile. Finally, pharmacological antihypertensive therapy was compared between subjects with hypertension vs. hypertension plus atherothrombotic disease using a Chi squared test.

We enrolled 341 subjects, with a mean age of 58 ± 13 years, predominantly woman (64.2%). From total sample, 24.3% ($n = 83$) had not hypertension, 63% ($n = 215$) had hypertension, and 12.6% ($n = 43$) had hypertension with history of atherothrombotic disease. Demographic, clinical, and biochemical data were compared between all three groups (Table 1). We observed statistical difference in age ($p < 0.0001$), gender ($p = 0.03$), SBP ($p < 0.0001$), DBP ($p < 0.0001$), dyslipidemia ($p = 0.01$), FPG ($p = 0.03$), total cholesterol ($p = 0.01$), and LDL-c ($p = 0.02$).

When we compared the vWF and PAI-1 antigen plasma levels, we observed higher vWF plasma levels in the group of patients with hypertension with history of atherothrombotic disease 102 ± 71.7 ng/mL ($p < 0.0001$), followed by patients with hypertension alone 89.9 ± 53.7 ng/mL ($p < 0.0001$), in comparison with the reference group 50.4 ± 33 ng/mL. The

PAI-1 plasma levels were higher in patients with hypertension alone 356.1 ± 275.8 ng/mL ($p = 0.04$), and similar in patients with hypertension and history of atherothrombotic disease 296.7 ± 288.9 ng/mL ($p = 0.84$) when compared with the reference group 286.9 ± 231.9 ng/mL (Fig. 1).

The metabolic syndrome components that significantly correlated with vWF were hypertension ($p \leq 0.001$), age ($p \leq 0.0001$), FPG ($p \leq 0.0001$), and SBP ($p = 0.006$) and those significantly correlated with PAI-1 were FPG ($p \leq 0.0001$), triglycerides ($p \leq 0.0001$), BMI ($p = 0.003$), HbA1c ($p = 0.006$) and age **($p = 0.0001$)** (Table 2). There was no correlation between gender and vWF ($p = 0.59$) and PAI-1 ($p = 0.89$) levels (Table 2).

An ANCOVA was performed to explain the variation in vWF plasma levels with hypertension, age, FPG, and SBP as explanatory variables. The model explained only 18% of variability in vWF plasma levels (F-statistic < 0.0001). Hypertension ($p = 0.0001$), age ($p < 0.0001$) and FPG ($p = 0.001$) demonstrated statistical significance (Table 3). Moreover, the effect of hypertension, age and FPG increased for individuals with higher vWF plasma levels at 0.25, 0.50 and 0.75 quantiles. Hypertension, age and FPG allow us to explain more than 26%, 70% and 86% of the variability of vWF plasma levels at 0.25, 0.50 and 0.75 quantiles, respectively (Table 3). The vWF plasma values levels observed of each of those quantiles were as followed: 0.25 (40.7 ng/mL), 0.50 (64.9 ng/mL), 0.75 (106.8 ng/mL).

An MLR analysis was performed to explain the variation of PAI-1 plasma levels attributed to variation in FPG, triglycerides, BMI, HbA1c, and age. The model explained only 11% of variability in PAI-1 plasma levels (F-statistic < 0.0001). FPG, triglycerides, BMI, HbA1c and age showed statistical significance, with an inverse relationship between age and PAI-1 (Table 4). Moreover, the effect of FPG, triglycerides and HbA1c increased for individuals with higher PAI-1 plasma levels at 0.25, 0.50 and 0.75 quantiles, while the effect of age decreased for individuals with higher PAI-1 plasma concentration at 0.25 and 0.50 quantiles. FPG, triglycerides, HbA1c and age allow us to explain more than 66%, 73% and 90% of the variability of PAI-1 plasma levels for 0.25, 0.50 and 0.75 quantiles, respectively (Table 4). The PAI-1 plasma levels observed of each of those quantiles were as followed 0.25 quantile (130.1 ng/mL), 0.50 quantile (238.9 ng/mL), 0.75 quantile (468.8 ng/mL).

When we performed a MLR analysis, the variables included in the model explained a small fraction of the variability in vWF and PAI-1 plasma levels. However, when a quantile regression approach was applied, the model explained up to 86% of the variability of vWF from 0.75 quantiles, and up to 90% of the variability of PAI-1 from 0.75 quantiles.

Table 1 Clinical and Epidemiological characteristics between controls, subjects with hypertension, and subjects with hypertension plus history of atherothrombotic disease

Variable	Total sample $n = 341$	Reference group $n = 83$	Hypertension $n = 215$	Hypertension plus ATD $n = 43$	p value
Demographic					
Age, years ± SD	58 ± 13	48 ± 14	61 ± 10	63 ± 12	< 0.0001
Woman, n (%)	219 (64.2)	54 (65)	145 (67.4)	20 (46.5)	0.03
Clinical					
BMI, kg/m^2 ± SD	28.9 ± 4.7	29.1 ± 5.7	28.8 ± 4.3	29.2 ± 5.2	0.97
SBP, mmHg ± SD	130.6 ± 19.6	115 ± 13.2	136.1 ± 19.2	132.7 ± 18.3	< 0.0001
DBP, mmHg ± SD	81.2 ± 11.4	75.8 ± 9.1	83.4 ± 11.1	80.3 ± 13.7	< 0.0001
Dyslipidemia, n (%)	181 (53)	36 (43.9)	74 (61.6)	11 (37.9)	0.01
Smoking status, n (%)	74 (21.7)	21 (25)	43 (20)	10 (23)	0.58
Biochemical					
FPG, mg/dL ± SD	88.7 ± 9.3	85.9 ± 9.2	89.4 ± 9.4	90.3 ± 10.6	0.03
HbA1c, % ± SD	5.6 ± 0.4	5.5 ± 0.4	5.6 ± 0.4	5.7 ± 0.3	0.16
Total Cholesterol, mg/dL ± SD	187 ± 37	184.8 ±44.2	190.7 ± 35.8	172.2 ± 42.8	0.01
LDL-c, mg/dL ± SD	108.2 ± 32.7	107.7 ± 37.7	110.9 ± 32.1	95.7 ± 36.8	0.02
HDL-c, mg/dL ± SD	47 ± 10.1	46.4 ± 10.7	47.7 ± 10.7	44.9 ± 9.7	0.18
Triglycerides, mg/dL ± SD	161.1 ± 91.8	157.5 ± 96.2	163.7 ± 98.2	154.7 ± 78.5	0.80
vWF, ng/mL ± SD	81.8 ± 55.4	50.4 ± 33	89.9 ± 53.7	102 ± 71.7	< 0.001
PAI-1, ng/mL ± SD	331.8 ± 269.4	286.9 ± 231.9	356.1 ± 275.8	296.7 ± 288.9	< 0.001

ATD atherothrombotic disease, *BMI* body mass index, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *HbA1c* glycated hemoglobin, *HDL-c* high density lipoprotein cholesterol, *LDL-c* low density lipoprotein cholesterol, *PAI-1* plasminogen activator inhibitor type 1, *SBP* systolic blood pressure, *SD* standard deviations, *vWF* von Willebrand Factor

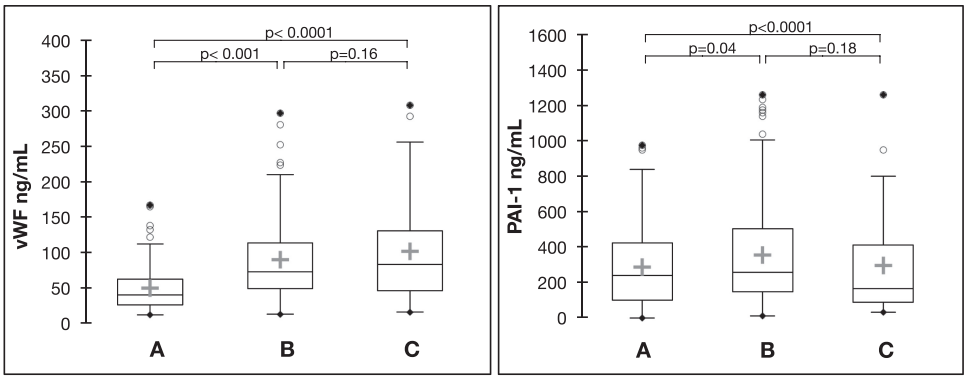


Fig. 1 vWF and PAI-1 antigen plasma levels comparison between controls (**A**), subjects with hypertension (**B**), and patients with hypertension plus history of atherothrombotic disease (**C**). p: *p* value; PAI-1: plasminogen activator inhibitor type 1; vWF: von Willebrand factor + : mean

Table 2 Correlation coefficients between vWF and PAI-1 plasma levels with components of the metabolic syndrome in a sample of 341 subjects

Variable	vWF	*p* value	PAI-1	*p* value
Continuous				
Age	0.34	< 0.0001	-0.13	0.0001
BMI	0.07	0.21	0.16	0.003
SBP	0.15	0.006	0.02	0.67
DBP	0.06	0.25	0.11	0.06
FPG	0.23	< 0.0001	0.20	< 0.0001
HbA1c	-0.008	0.88	0.15	0.006
Total cholesterol	0.06	0.30	0.09	0.11
LDL-c	0.05	0.32	0.02	0.64
HDL-c	0.01	0.85	-0.07	0.17
Triglycerides	-0.04	0.49	0.19	< 0.0001
Categorical				
Woman	-0.03	0.59	-0.008	0.89
Smoking status	-0.07	0.21	0.005	0.93
Dyslipidemia	-0.08	0.14	0.05	0.32
Hypertension	0.38	< 0.0001	0.08	0.13
Hypertension + ATD	0.10	0.06	-0.10	0.08

ATD atherothrombotic disease, *BMI* body mass index, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *HbA1c* glycated hemoglobin, *HDL-c* high density lipoprotein cholesterol, *LDL-c* low density lipoprotein cholesterol, *PAI-1* plasminogen activator inhibitor type 1, *SBP* systolic blood pressure, *vWF* von Willebrand Factor

When a quantile regression model was performed to explore the relationship between independent variables and conditional quantiles of vWF (Fig. 2), the quantile coefficients for hypertension, age and FPG on vWF plasma levels were significantly different from the MLR coefficients. When a quantile regression model was performed (Fig. 2), the quantile coefficients for FPG, triglycerides, HbA1c and age on PAI-1 plasma levels were significantly different from the MLR coefficients.

Table 5 shows the antihypertensive treatment characteristics in subjects with hypertension vs. hypertension plus atherothrombotic disease. The angiotensin II receptor blocker (ARB) antihypertensive treatment was the most frequently used in both groups of patients with and without atherothrombotic disease (56.2% and 46.5%, $p = 0.24$) and the less frequent was calcium channel blocker 14.3% vs. 27.9% ($p = 0.04$), respectively. In the group of hypertensive patients, the monotherapy was used in more than 51.6% of cases, whereas in the group of hypertension plus atherothrombotic disease only one antihypertensive medicine was administrated in 46.5% of the cases ($p = 0.61$). The combination of antihypertensive drugs was used in 37.2% and 46.5% in the group of hypertension and hypertension and atherothrombotic disease, respectively (Table 5). There was a statistically significant higher frequency of calcium channel blocker (27.9% vs. 14.4%, $p = 0.04$) and β-blocker (34.8% vs. 17.6%, $p = 0.02$) therapy in the group of patients with hypertension plus atherothrombotic disease vs. hypertension only, respectively.

Table 6 shows the comparison of vWF and PAI-1 antigen plasma levels according to type of antihypertensive pharmacotherapy and number of antihypertensive drugs in hypertensive subjects with and without ATD. We observed the lowest plasma levels of vWF in patients with ARB´S monotherapy treatment, and the lowest levels of PAI-1 plasma antigens in patients who received calcium channel blocker monotherapy treatment. The lowest levels of both vWF and PAI-1 were identified in the group of patients who were treated with the combinations of three antihypertensive drugs.

Graphical Abstract, vWF and PAI-1 levels are elevated in patients with hypertension, probably due to uncontrolled blood pressure and inadequate antihypertensive therapy.

Discussion

Endothelial dysfunction is present in patients with hypertension, which is a risk factor for atherothrombotic disease. Earlier studies had demonstrated that von Willebrand Factor (vWF) represents a biomarker for endothelial damage/dysfunction [10]. In our knowledge, this is the first study in Mexican population 1) To determine and compared the

Table 3 Model explaining variation in vWF plasma levels attributed to variation in explanatory variables using two different approaches: ANCOVA and quantile regression analysis

Explanatory Variables	ANCOVA		Quantile Regression		
	β (95% CI)	p value	β (95% CI)		
			at 0.25 quantile	at 0.50 quantile	at 0.75 quantile
Hypertension	0.22 (0.34 to 0.11)	0.0001	19 (17 to 21)	32 (31 to 34)	35 (34 to 37)
Age	0.2 (0.1 to 0.3)	< 0.0001	0.5 (0.4 to 0.6)	0.6 (0.5 to 0.6)	1.0 (1 to 1.1)
FPG	0.2 (0.08 to 0.28)	0.001	0.2 (0.08 to 0.24)	0.7 (0.68 to 0.78)	1.5 (1.4 to 1.5)
SBP	-0.07 (-0.19 to 0.04)	0.19	-0.2 (-0.28 to -0.2)	-0.09 (-0.1 to -0.06)	-0.2 (-0.28 to -0.2)
R^2	0.18		0.26	0.70	0.86
F-statistic	< 0.0001		< 0.0001	< 0.0001	< 0.0001

β standardized correlation coefficient (95% confidence interval), *FPG* fasting plasma glucose, R^2 determination coefficient represents percentage of variance explained by the explanatory variables of the model, *SBP* systolic blood pressure, *vWF* von Willebrand factor

Table 4 Model explaining variation in PAI-1 plasma levels attributed to variation in explanatory variables using two different approaches: MLR and quantile regression analysis

Explanatory variables	MLR		Quantile Regression		
	β (95% CI)	p value	β (95% CI)		
			at 0.25 quantile	at 0.50 quantile	at 0.75 quantile
FPG	0.14 (0.03 to 0.25)	0.01	3.9 (3.4 to 4.5)	3.4 (3 to 3.7)	7 (6.8 to 7.2)
Triglycerides	0.15 (0.05 to 0.26)	0.004	0.2 (0.17 to 0.3)	0.33 (0.3 to 0.36)	0.7 (0.65 to 0.69)
BMI	0.09 (-0.01 to 0.20)	0.08	3 (2 to 4)	4 (3.5 to 4.7)	12 (11 to 12)
HbA1c	0.12 (0.01 to 0.23)	0.02	35 (24 to 46)	56 (49 to 63)	71 (66 to 75)
Age	-0.15 (-0.25 to -0.04)	0.006	-2 (-2.5 to -1.7)	-3 (-3.2 to -2.7)	-3 (-3.2 to -2.9)
R^2	0.11		0.66	0.73	0.90
F-statistic	< 0.0001		< 0.0001	< 0.0001	< 0.0001

β standardized correlation coefficient (95% confidence interval), *BMI* body mass index, *FPG* fasting plasma glucose, *HbA1c* glycated hemoglobin, *MLR* multivariate linear regression, *PAI-1* plasminogen activator inhibitor type 1, R^2 determination coefficient represents percentage of variance explained by the explanatory variables of the model

plasma concentration of vWF and PAI-1 between normotensive individuals and hypertensive patients with and without atherothrombotic disease, 2) To identify the variables of study are associated with the variation of the plasmatic levels of vWF and PAI-1, and 3) To evaluate the influence of antihypertensive treatment on the vWF and PAI-1 plasma concentration. vWF and PAI-1 levels between normotensive subjects and hypertensive patients with and without atherothrombotic disease, and to evaluate the influence of antihypertensive medication on those biomarkers. We found the highest concentration of vWF in hypertensive individuals with atherothrombotic disease (102 ± 71.7 ng/mL) compared to patients with hypertension without atherothrombotic disease (89.9 ± 53.7 ng/mL) or normotensive individuals (50.4 ± 33 ng/mL) ($p < 0.0001$), (Table 1). The increased vWF plasma level was significantly correlated with SBP ($r = 0.15$, $p = 0.006$), (Table 2) but not with DBP ($r = 0.06$, $p = 0.25$) (Table 2). Our results are in line with those published by Blann et al., [11] whom demonstrated that high vWF levels correlated with SBP ($r = 0.42$, $p < 0.0002$), but in contrast to our results, they also found a correlation between vWF and DBP ($r = 0.25$, $p < 0.05$). Moreover, Lip et al., demonstrated higher vWF in patients with hypertension, compared with normotensive healthy controls [12]. Previous studies found that a hypercoagulable state is present in patients with hypertension and this state was associated with increased blood pressure and endothelium-dependent vasodilation impairment [13]. Our results indicate a possible association between vWF and endothelial damage, which precedes a vascular thrombotic complication in this type of patients, with long time high blood pressure exposure. We propose that a good control of blood pressure is needed to avoid chronic exposure of high pressure on vessels.

In our study, hypertension, age and FPG allow us to explain more than 26%, 70% and 86% of the variability of vWF plasma levels at 0.25, 0.50 and 0.75 quantiles, respectively, being hypertension the variable with the highest β-coefficient to determine the variability of vWF,

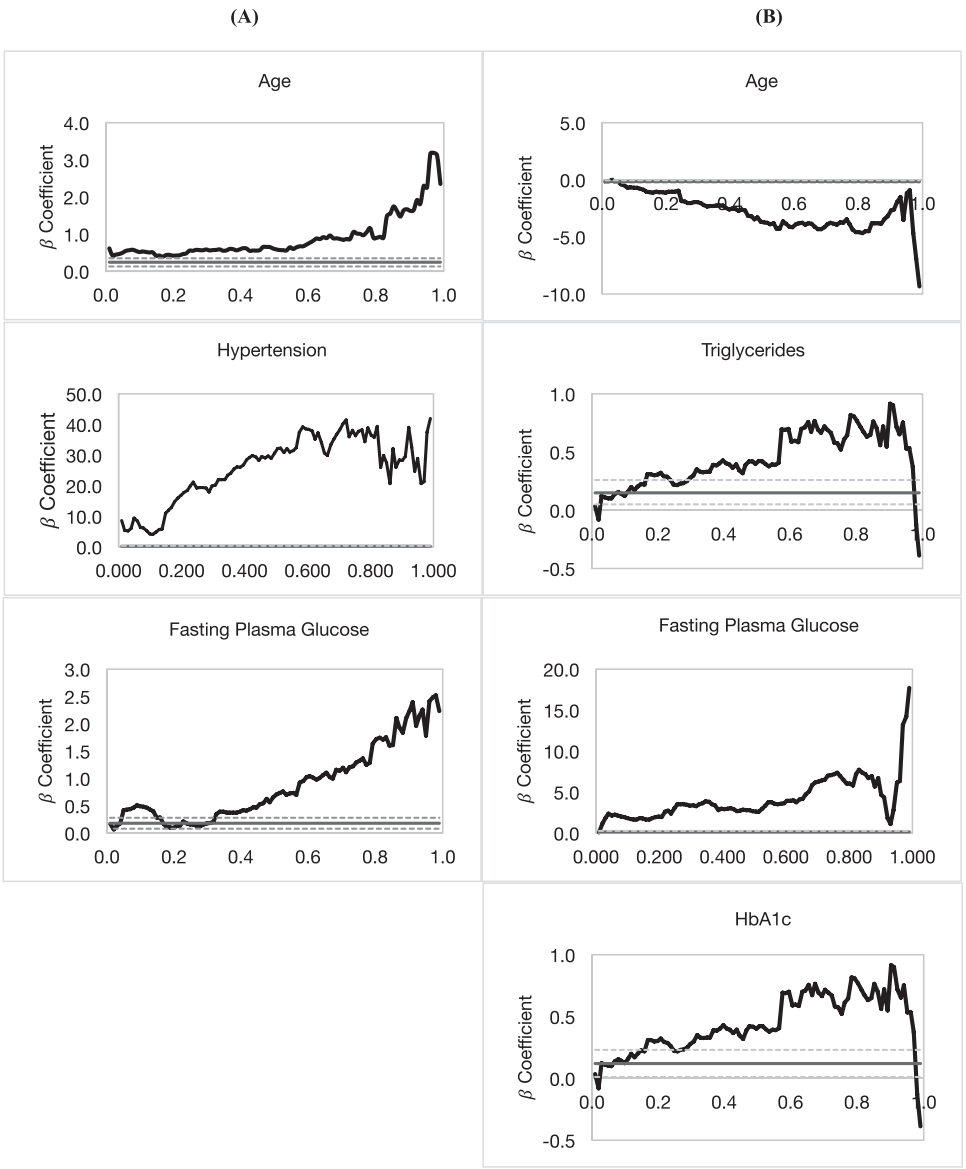


Fig. 2 Quantile process charts for vWF (**A**) and PAI-1 (**B**). Gray dotted line: 95% confidence interval; HbA1c: glycated hemoglobin; horizontal axis: quantiles; red line: multivariate linear regression coefficient

even more than any other biochemical parameter (Table 3). Therefore, our study demonstrated that hypertension is associated with increased levels of vWF and endothelial damage, which play an important role in the pathophysiology of atherothrombotic disease [14]. Previous studies in vivo and in vitro showed that haemodynamic forces significantly influence the gene expression and phenotype of vascular cells that induce functional changes in response to mechanical stress, which can produce: inflammation, changes in the vascular cell adhesion, endothelial cell proliferation and apoptosis, vascular smooth muscle cell differentiation, proliferation and migration, oxidative stress, endothelial cell procoagulant properties and promoting atherogenesis [15, 16].

Moreover, we found a significantly correlation between older age and increased vWF plasma levels ($r = 0.34$, $p < 0.0001$) (Table 2). Our results are similar to those demonstrated by Lip et al., with a positive correlation between high levels of vWF and age ($r = 0.31$, $p < 0.001$) [12]. The association between increased levels of vWF and older hypertensive individuals might suggest a longtime exposure of blood vessels to high hemodynamic forces indicating a progressive endothelial cell damage/dysfunction [17].

In the present study, we evaluate hypertensive individuals without T2 Diabetes Mellitus. However, we found a positive correlation between vWF plasma levels and FPG ($r = 0.23$, $p < 0.0001$) (Table 3). It has been shown an association between high concentration of vWF and increasing insulin levels suggesting a relationship of vWF with insulin resistance and the metabolic syndrome [18]. Earlier studies proposed that endothelial damage/dysfunction maybe a central component of the metabolic syndrome, possibly

Table 5 Antihypertensive treatment characteristics in subjects with hypertension vs. hypertension plus atherothrombotic disease

Characteristic	Hypertension $n=215$	Hypertension plus ATD $n=43$	*p* value
Time from diagnosis, years [IQR]	8 [3–15]	10 [6–17.5]	0.04
Antihypertensive action mechanism, n (%)			
ARB	121 (56.2)	20 (46.5)	0.24
ACE inhibitor	51 (23.7)	13 (30.2)	0.43
Diuretic	50 (23.2)	7 (16.2)	0.42
BB	38 (17.6)	15 (34.8)	0.02
CCB	31 (14.4)	12 (27.9)	0.04
Number of antihypertensive drugs, n (%)			
0	24 (11.2)	3 (6.9)	0.58
1	111 (51.6)	20 (46.5)	0.61
2	60 (27.9)	14 (32.5)	0.58
3	20 (9.3)	5 (11.6)	0.58
4	0 (0)	1 (2.3)	-
Type of antihypertensive pharmacotherapy, n (%)			
None	24 (11.2)	3 (6.9)	0.58
ARB monotherapy	60 (27.9)	10 (23.2)	0.57
ACE inhibitor monotherapy	37 (17.2)	3 (6.9)	0.10
CCB monotherapy	7 (3.2)	4 (9.3)	0.09
BB monotherapy	7 (3.2)	3 (6.9)	0.37
Combined	80 (37.3)	20 (46.5)	0.30

ACE angiotensin converting enzyme, *ARB* angiotensin II receptor blocker, *ATD* atherothrombotic disease, *BB* β-blocker, *CCB* calcium channel blocker, *IQR* interquartile range

Table 6 Comparison of vWF and PAI-1 antigen plasma levels according to type of antihypertensive pharmacotherapy and number of antihypertensive drugs in hypertensive subjects with and without atherothrombotic disease

	Type of antihypertensive pharmacotherapy						*p* value
	Monotherapy					Combined	
	ACE inhibitors $n=40$		ARBs $n=70$	CCB $n=11$	BB $n=10$	$n=100$	
vWF (ng/mL)	68 [47–112]		64 [46–95]	85 [61–153]	83 [53–99]	95 [56–137]	**0.09**
PAI-1 ng/mL	329 [177–656]		236 [128–502]	163 [114–189]	226 [166–329]	232 [148–459]	**0.37**
	Number of antihypertensive drugs						
	0 $n=27$	1 $n=131$		2 $n=74$	3 $n=25$	4 $n=1$	
vWF	74 [46–113]	69 [47–104]		96 [61–138]	66 [54–114]	131	0.07
PAI-1	262 [149–515]	236 [140–518]		268 [147–498]	198 [149–285]	177	0.44

ACE angiotensin converting enzyme, *ARB* angiotensin II receptor blocker, *BB* β-blocker, *CCB* calcium channel blocker, *PAI-1* plasminogen activator inhibitor type 1, *vWF* von Willebrand factor

preceding or even causing its development [19]. However, Lim et al., [20] demonstrated in hypertensive patients who developed metabolic syndrome that vWF plasma levels were higher and independently associated with the presence of established metabolic syndrome and increased with the number of components. Their results suggested that endothelial damage/dysfunction may be a consequence, rather that the cause of the metabolic syndrome. Our results, demonstrated that in hypertensive patients the vWF concentration could be positive regulated by glucose or lipids levels, even without the presence of Type 2 diabetes mellitus or metabolic syndrome. Therefore, a point of interest will be to explore the endothelial mechanism and possible interaction between hypertension and Type 2 diabetes or other metabolic components in the same individual to prevent the disease development.

When we analyzed the vWF plasma levels, we observed the highest concentration in patients with calcium channel

blockers treatment (85 [61–153 U/dL]) (Table 6). Clinical studies had demonstrated that plasma levels of vWF possible be diminished by some antihypertensive medication [9, 21]. Although, we found the lowest plasma levels of vWF in patients who were treated with angiotensin II receptor blocker medication (64 [46–95 ng/mL]) (Table 6), the levels where higher to those observed in normotensive individuals (50.4 ± 33 ng/mL). Therefore, our results suggest that antihypertensive monotherapy has not enough influence on lowering vWF, which was increased by long time high blood pressure exposure and this reflects endothelial damage dysfunction, which play an important role in the thrombosis development. Therefore, we consider that earlier diagnosis and adequate control of blood pressure is essential to avoid endothelial damage and thrombotic complications. We propose that plasma levels of vWF should be determined in this type of patients with specific focus in those with no good control or resistance blood pressure.

In the present study, there was no significant correlation between gender (woman) and vWF (r = 0.03, $p = 0.59$). Our results are in contrast to those published by Mansfield et al., [22] who demonstrated that high levels of vWF were higher in women than in male $p < 0.001$.

Plasminogen antigen inhibitor type 1 (PAI-1) is a major regulator of the fibrinolytic system, and high level is associated with atherothrombotic disease. Increased PAI-1 levels were correlated with glucose, triglycerides, HbA1c, explaining 66%, 73% and 90% of variability. In contrast, lower PAI-1 concentration was determined by older age (r = 0.13, $p = 0.0001$) (Table 3).

We found a higher PAI-1 plasma levels in hypertensive patients without atherothrombotic disease (356.1 ± 275.8 ng/mL), and similar in patients with hypertension and atherothrombotic disease (296.7 ± 288.9) when compared with the reference group (286.9 ± 231.9 ng/mL) (< 0.001) (Table 1). A possible explanation for similar PAI-1 levels between hypertensive patients with hypertension and atherothrombotic disease and reference group, might be the effect of pharmacotherapy, adherence to treatment as well as dietary hygiene measures on reduction of PAI-1 concentration, which were beyond the aim of the present research. Our results demonstrated no correlation between gender (female) and PAI-1 plasma levels (r = 0.13, $P = 0.89$). In contrast, Mansfield and coworkers demonstarted, an association between increased levels of PAI-1 and gender (female) [22].

Contrary to the effect of high blood pressure on vWF, we observed that SBP (r = 0.02, $p = 0.67$) and DBP (r = 0.11, $p = 0.06$) were not correlated with PAI-1 levels. In contrast, Jacobs et al., [7] found that PAI-1 activity correlates with SBP and DBP and this represented an increased risk for thrombotic complications. Also, Lip et al., [12] demonstrated higher PAI-1 levels in patients with hypertension, compared with normotensive healthy controls.

Moreover, we found a significantly correlation between glucose (r = 0.20, $p < 0.0001$), HbA1c (r = 0.15, $p = 0.006$) and triglycerides (r = 0.19, $p < 0.001$) and increased PAI-1 plasma levels (Table 2).

PAI-1 levels can be regulated by several factors and two of them are relevant in modifying cardiovascular risk such the activity of the renin–angiotensin–aldosterone system (RAAS) and insulin resistance [23]. Moreover, aldosterone stimulates PAI-1 expression in vitro, and PAI-1 levels correlate closely with aldosterone levels in normal human subjects. Injured/damage endothelial cells and an activated RAAS accompanied by high blood pressure could contribute to the hypercoagulable status and in the promotion of thrombosis in hypertensive patients [24].

When we analyzed the PAI-1 plasma levels related with the antihypertensive medication, we found the highest level of PAI-1 in patients with angiotensin converting enzyme inhibitors therapy (329 [177–656 ng/mL]) and the lowest plasma levels of PAI-1 (163 [114–189 ng/mL]) in patients who were treated with calcium channel blocker. (Table 6) Some studies have shown that group of calcium channel blockers, the dihydropyridines affect the fibrinolytic system by increasing plasma t-PA activity [25]. Because both PAI-1 and t-PA are synthetized in the vascular endothelium and endothelial dysfunction induces an imbalance in fibrinolysis, improving endothelial function might reverse the fibrinolytic imbalance. The calcium channel blocker had been shown to improve endothelial function through their antioxidative properties and effects on nitric oxide synthase expression and activity [25]. More studies are needed to evaluate the effect of calcium channel blocker on reduction of PAI-1 plasma levels.

When we analyzed the antihypertensive therapy, we found that angiotensin II receptor blocker treatment was the antihypertensive drug more frequently used and the calcium channel blocker was the less common indicated in patients with and without atherothrombotic disease complications. (Table 5) Our results are similar to those published by Yang et al., in a meta- analysis that included 1.1 million patients who received angiotensin II receptor blocker prescription for antihypertensive therapy in China, [26] but calcium channel blocker also was the other drug most frequently used for treatment. In the present study the combination of two or more drugs was the most frequent therapeutic used (Table 5).

When we analyzed the vWF and PA1-1 plasma levels and the number of antihypertensive drugs, we found the lowest plasma levels of vWF 66 [54–114 ng/mL] and PAI-1 196 [149–286 ng/mL] (Table 6) in patients who were treated with three or more antihypertensive drugs. In contrast the highest level of PAI-1 was observed in patients with no treatment or only one drug medication. Although, there was no differences related to vWF and PAI-1 plasma

levels between the type of antihypertensive pharmacotherapy [vWF $p = 0.09$, PAI-1 ($p = 0.37$)], or number of antihypertensive drugs [vWF $p = 0.07$, PAI-1 ($p = 0.44$)] (Table 6), we consider that should be evaluate two or more drug antihypertensive treatment not only to obtain a good control blood pressure, but as well vWF and PAI-1 plasma concentration to avoid thrombotic complications.

One limitation was the size of the sample. However, the number of the patients and controls included in this study allowed us to demonstrated the highest plasma levels of vWF in patients with hypertension and atherothrombotic disease, meanwhile the highest concentration of PAI-1 was present in hypertensive patients without thrombotic disease, and those findings could be correlated with endothelial dysfunction and an hypofibrinolytic state respectively in those patients. More studies with larger number of patients are needed to corroborate our results.

Conclusions

This is the first study in Mexican population to compare the vWF and PAI-1 levels in hypertensive patients without T2 Diabetes Mellitus and evaluate the effect on the antihypertensive medication on the regulation of those biomarkers. In the present study, the highest plasma levels of vWF were found in hypertensive patients with atherothrombotic disease and for PAI-1 levels were in hypertensive patients without thrombotic complications. Hypertension, age and FPG determine up to 86% of variability of vWF plasma levels, while FPG, HbA1c, triglycerides and age determine up to 90% of the variability of PAI-1 plasma concentration. Although, hypertensive patients treated with angiotensin II receptor blocker had the lowest levels of vWF and the patients treated with calcium channel blocker therapy had the lowest levels of PAI-1, none had normal levels of those biomarkers. The highest PAI-1 concentration was observed in hypertensive patients, whom were treated with only one drug or no medication. In contrast, we observed the lowest levels of vWF and PAI-1 concentration in patients who were treated with 3 or more antihypertensive drugs. Those results reinforce the importance of good blood pressure and metabolic components control, as well as to find the right combination of two or more drugs in patients with hypertension at earlier stages of the disease to avoid thrombotic complications such as myocardial infarction or stroke. In the present study, we demonstrated that endothelial dysfunction and hypofibrinolytic state are two mechanisms that contribute to the pathophysiology of thrombosis in patients with hypertension. Therefore, we consider an important to obtain a good blood pressure control, to find the right therapeutic drug combination and to determine the vWF and PAI-1 levels to detect a possible endothelial dysfunction and a decreased fibrinolytic activity to avoid thrombotic complications.

Future Perspectives: We demonstrated that monotherapy is not sufficient to obtain a good control of blood pressure and normal levels of vWF and PAI-1. Therefore, we consider that more studies with greater number of patients are needed to evaluate the specific contribution of angiotensin II receptor blocker and calcium channel blocker medication on lowering vWF and PAI-1 levels respectively, and to determine the specific combination of two or more drugs for each hypertensive patient to control those parameters. We suggest that vWF and PAI-1 determination should be included in patients with hypertension to monitoring a possible endothelial damage as well as hypofibrinolytic stage to avoid thrombotic complications such myocardial infarction and/or stroke.

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Data Availability No Data associated in the manuscript.

Declarations

Ethical approval The study protocol was reviewed and approved by the Human Ethical Committee, and Medical Research Council of the Instituto Mexicano Del Seguro Social, and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Informed written consent was obtained from all subjects before enrollment.

Competing interest There was no competing interest.

References

1. Verdecchia P, Reboldi G, Angeli F. The 2020 International Society of Hypertension global hypertension practice guide-lines-key messages and clinical considerations. Eur J Intern Med. 2020;82:1–6. https://doi.org/10.1016/j.ejim.2020.09.001.
2. Pistoia F, Sacco S, Degan D, Tiseo C, Ornello R, Carolei A. Hypertension and Stroke: Epidemiological Aspects and Clinical Evaluation. High Blood Press Cardiovasc Prev. 2016;2:9–18. https://doi.org/10.1007/s40292-015-0115-2.
3. Gragnano F, Sperlongano S, Golia E, Natale F, Bianchi R, Crisci M, Fimiani F, Pariggiano I, Diana V, Carbone A, Cesaro A, Concilio C, Limongelli G, Russo M, Calabrò P. The Role of von Willebrand Factor in Vascular Inflammation: From Pathogenesis to Targeted Therapy. Mediators Inflamm. 2017;2017:5620314. https://doi.org/10.1155/2017/5620314.
4. Li Q, Youn JY, Cai H. Mechanisms and consequences of endothelial nitric oxide synthase dysfunction in hypertension. J Hypertens. 2015;33:1128–36. https://doi.org/10.1097/HJH.0000000000000587.
5. Liakos CI, Vyssoulis GP, Michaelides AP, Chatzistamatiou EI, Theodosiades G, Toutouza MG, Markou MI, Synetos AG, Kallikazaros IE, Stefanadis CI. The effects of angiotensin receptor blockers vs. calcium channel blockers on the acute exercise-induced inflammatory and thrombotic response. Hypertens Res. 2012;35:1193–200. https://doi.org/10.1038/hr.2012.134.

6. Mertens I, Verrijken A, Michiels JJ, Van der Planken M, Ruige JB, Van Gaal LF. Among inflammation and coagulation markers, PAI-1 is a true component of the metabolic syndrome. Int J Obes (Lond). 2006;30:1308–14. https://doi.org/10.1038/sj.ijo.0803189.
7. Jacobs A, Schutte AE, Ricci C, Pieters M. Plasminogen activator inhibitor-1 activity and the 4G/5G polymorphism are prospectively associated with blood pressure and hypertension status. J Hypertens. 2019;37:2361–70. https://doi.org/10.1097/HJH.0000000000002204.
8. Tabak O, Gelisgen R, Uzun H, Kalender B, Balci H, Curgunlu A, Simsek G, Karter Y, Simsek G, Karter Y. Hypertension and hemostatic/fibrinolytic balance disorders. Clin Invest Med. 2009;32:285–90. https://doi.org/10.25011/cim.v32i6.10664.
9. Hirschl MM, Bur A, Woisetschlaeger C, Derhaschnig U, Laggner AN. Effects of candesartan and lisinopril on the fibrinolytic system in hypertensive patients. J Clin Hypertens (Greenwich). 2007;9:430–5. https://doi.org/10.1111/j.1524-6175.2007.06506.x.
10. Konukoglu D, Uzun H. Endothelial Dysfunction and Hypertension. Adv Exp Med Biol. 2017;956:511–40. https://doi.org/10.1007/5584-2016-90.
11. Blann AD, Naqvi T, Waite M, McCollum CN. von Willebrand factor and endothelial damage in essential hypertension. J Hum Hypertens. 1993;7:107–11.
12. Lip GY, Blann AD, Jones AF, Lip PL, Beevers DG. Relation of endothelium, thrombogenesis, and hemorheology in systemic hypertension to ethnicity and left ventricular hypertrophy. Am J Cardiol. 1997;80:1566–71. https://doi.org/10.1016/s0002-9149(97)00749-2.
13. Yang P, Liu YF, Yang L, Wei Q, Zeng H. Mechanism and Clinical Significance of the Prothrombotic State in Patients with Essential Hypertension. Clin Cardiol. 2010;33(E81–6):E81–6. https://doi.org/10.1002/clc.20719.
14. Souilhol C, Serbanovic-Canic J, Fragiadaki M, Chico TJ, Ridger V, Roddie H, Evans PC. Endothelial responses to shear stress in atherosclerosis: a novel role for developmental genes. Nat Rev Cardiol. 2020;17:52–63. https://doi.org/10.1038/s41569-019-0239-5.
15. Chistiakov DA, Orekhov AN, Bobryshev YV. Effects of shear stress on endothelial cells: go with the flow. Acta Physiol (Oxf). 2017;219:382–408. https://doi.org/10.1111/apha.12725.
16. Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. Biomed Res Int. 2014;2014:406960. https://doi.org/10.1155/2014/406960.
17. Biguzzi E, Castelli F, Lijfering WM, Cannegieter SC, Eikenboom J, Rosendaal FR, van Hylckama VA. Rise of levels of von Willebrand factor and factor VIII with age: Role of genetic and acquired risk factors. Thromb Res. 2020;197:172–8. https://doi.org/10.1016/j.thromres.2020.11.016.
18. Spencer CG, Felmeden DC, Blann AD, Lip GY. Haemorheological, platelet and endothelial indices in relation to global measures of cardiovascular risk in hypertensive patients: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial. J Intern Med. 2007;261:82–90. https://doi.org/10.1111/j.1365-2796.2006.01735.x.
19 Grandl G, Wolfrum C. Hemostasis, endothelial stress, inflammation, and the metabolic syndrome. Semin Immunopathol. 2018;40(2):215–24. https://doi.org/10.1007/s00281-017-0666-5.
20. Lim HS, Lip GYH, Blann AD. Plasma von Willebrand factor and the development of the metabolic syndrome in patients with hypertension. J Clin Endocrinol Metab. 2004;89:5377–81. https://doi.org/10.1210/jc.2004-0616.
21. Karaman M, Balta S, Seyit Ahmet AY, Cakar M, Naharci I, Demirkol S, Celik T, Arslan Z, Kurt O, Kocak N, Sarlak H, Demirbas S, Bulucu F, Bozoglu E. The comparative effects of valsartan and amlodipine on vWf levels and N/L ratio in patients with newly diagnosed hypertension. Clin Exp Hypertens. 2013;35:516–22. https://doi.org/10.3109/10641963.2012.758734.
22. Mansfield MW, Heywood DM, Grant PJ. Sex differences in coagulation and fibrinolysis in white subjects with non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol. 1996;16:160–4. https://doi.org/10.1161/01.atv.16.1.160.
23. Wägner AM, Wiebe JC, Boronat M, Saavedra P, Marrero D, Varillas F, Nóvoa FJ. Insulin resistance explains the relationship between novel cardiovascular risk factors and hypertension. The Telde Study. J Endocrinol Invest. 2011;34:e409–12. https://doi.org/10.3275/7325.
24. Srikumar N, Brown NJ, Hopkins PN, Jeunemaitre X, Hunt SC, Vaughan DE, Williams GH. PAI-1 in human hypertension: relation to hypertensive groups. Am J Hypertens. 2002;15:683–90. https://doi.org/10.1016/s0895-7061(02)02952-7.
25. Taddei S, Virdis A, Ghiadoni L, Magagna A, Favilla S, Pompella A, Salvetti A. Restoration of nitric oxide availability after calcium antagonist treatment in essential hypertension. Hypertension. 2001;37:9432–48. https://doi.org/10.1161/01.hyp.37.3.943.
26. Yang R, Tang J, Kuang M, Liu H. Analysis of prescription status of Antihypertensive drugs in Chinese patients hypertension based on real-world study. Ann Med. 2023;55:276–84. https://doi.org/10.1080/07853890.2022.2162113.

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