Faculdade de Medicina de São José do Rio Preto
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Níveis de Metaloproteinase-9 e Parâmetros Hemodinâmicos Centrais em Diferentes Níveis de Pressão Arterial

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Orientador: Prof. Dr. José Fernando Vilela Martin

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SUMÁRIO

Aos meus pais Mário e Neuza pelo incentivo e apoio incondicional em todos os momentos, e que muitas vezes se doaram e renunciaram aos seus sonhos, para que eu pudesse realizar os meus.

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"Descobrir consiste em olhar para o que todo mundo

está vendo e pensar uma coisa diferente"

Roger Von Oech

Manuscrito 1

Manuscrito 3

Manuscrito 1

Manuscrito 2

Manuscrito 3

AI	*Augmentation index* (índice de incremento)
ANOVA	Análise de variância
CT	Colesterol total
DC	Débito cardíaco
DCV	Doenças cardiovasculares
FC	Frequência cardíaca
HAS	Hipertensão arterial sistêmica
HDL-c	Lipoproteína de alta densidade
HTC	Hipertenso controlado
HypUrg	Urgência hipertensiva
HypEmerg	Emergência hipertensiva
IMC	Índice de massa corporal
LDL-c	Lipoproteína de baixa densisdade
LOA	Lesões em órgão-alvo
MAPA	Monitorização ambulatorial da pressão arterial
MEC	Matriz extracelular
MMP	Metaloproteinase de matriz
OMS	Organização Mundial de Saúde
PA	Pressão arterial
PAS	Pressão arterial sistólica
PASc	Pressão arterial sistólica central
PAD	Pressão arterial diastólica
PADc	Pressão arterial diastólica central
PAM	Pressão arterial média

PH	Pré-hipertensão
PP	Pressão de pulso
RVP	Resistência vascular periférica
TIMP-1	Inibidor endógeno de metaloproteinase
TFGe	Taxa de filtração glomerular estimada
TG	Triglicérides
VOP	Velocidade de onda de pulso

RESUMO

Introdução: A pré-hipertensão predispõe à hipertensão arterial sistêmica (HAS), que é o principal fator de risco para o desenvolvimento de doenças cardiovasculares (DCV). Dentre os métodos de avaliação dos níveis pressóricos, a monitorização ambulatorial da pressão arterial (MAPA) destaca-se por registrar a pressão arterial (PA) durante 24 horas, bem como alterações em seu ciclo circadiano. Além dos parâmetros hemodinâmicos periféricos, a MAPA 24h também pode mostrar parâmetros hemodinâmicos centrais, como os que avaliam a rigidez arterial. Assim, apresenta variáveis que podem se correlacionar com eventos cardiovasculares. Adicionalmente, os níveis de metaloproteinase-9 (MMP-9), enzima que participa da degradação de componentes da matriz extracelular, também têm sido associados ao desenvolvimento de DCV e no processo de rigidez arterial. **Objetivos:** Os objetivos do presente estudo foram: 1. Comparar as variáveis hemodinâmicas periféricas e centrais obtidas pela MAPA em diferentes níveis de PA, de acordo com a classificação de normotensão, pré-hipertensão e hipertensão arterial, e verificar a existência de correlação entre as variáveis obtidas pela MAPA e MMP-9. 2. Avaliar os níveis de MMP-9 na elevação aguda da PA, representada pela crise hipertensiva. **Métodos:** Participaram do presente estudo indivíduos normotensos (NT), pré-hipertensos (PH) e hipertensos controlados (HTC). Na avaliação dos níveis de MMP-9 participaram também indivíduos em crise hipertensiva, divididos em urgência hipertensiva (UH) e emergência hipertensiva (EH). Todos os voluntários foram avaliados quanto às variáveis bioquímicas e, com exceção do estudo na crise hipertensiva, foram monitorados pela MAPA quanto a variáveis hemodinâmicas periféricas, tais como pressão arterial sistólica (PAS), pressão arterial diastólica (PAD), pressão arterial média (PAM), pressão de pulso (PP), débito cardíaco (DC), resistência

vascular periférica (RVP); e centrais, como PA sistólica central (PASc), PA diastólica central (PADc), *augmentation index* 75% (AI75%), e velocidade de onda de pulso (VOP). **Resultados:** O grupo PH apresentou diferença significativa em dois estudos quando comparados a HTC em relação às variáveis periféricas (PAS) e centrais (PASc e VOP). Também houve diferença nesses estudos entre PH e NT quanto à PAS, PASc e PADc. O uso da MAPA também permitiu identificar diferenças significantes no descenso noturno sistólico e diastólico entre PH e HTC. Em relação aos níveis de MMP-9 observou-se em um dos estudos níveis progressivamente mais elevados entre HTC, UH e EH. Em outro estudo, o grupo PH apresentou níveis de MMP-9 mais elevados que HTC. No estudo de correlação, identificou-se correlação positiva entre MMP-9 com o DC e RVP. **Conclusões:** Sugere-se que no estado pré-hipertensivo estão presentes alterações de parâmetros hemodinâmicos periféricos e centrais e, consequentemente, alterações funcionais e estruturais que predispõem ao desenvolvimento da hipertensão arterial. Além disso, os níveis de MMP-9 parecem ser influenciados pela terapia anti-hipertensiva, uma vez que hipertensos tratados apresentam menores níveis de MMP-9 do que indivíduos pré-hipertensos. No caso da crise hipertensiva, os níveis de MMP-9 mais elevados podem se constituir em um importante biomarcador na elevação aguda da pressão arterial.

Palavras-chave: Hipertensão arterial sistêmica, pré-hipertensão, metaloproteinases, rigidez arterial.

ABSTRACT

Introduction: Prehypertension predisposes individuals to systemic hypertension, which is the main risk factor for the development of cardiovascular diseases (CVD). Ambulatory blood pressure monitoring (ABPM) is an important method to assess blood pressure (BP) as it records levels during 24 hours, as well as changes related to the circadian cycle. In addition to peripheral hemodynamic parameters, 24h ABPM assesses central hemodynamic parameters, such as those linked to arterial stiffness. Thus, this examination presents several variables that correlate with cardiovascular events. Additionally, levels of metalloproteinase-9 (MMP-9), an enzyme involved in the degradation of extracellular matrix components, have been associated with the development of CVD and the arterial stiffness process. **Objectives:** The aims of the present study were: 1. to compare the peripheral and central hemodynamic variables obtained by ABPM at different BP levels according to the classification of normotension, prehypertension and arterial hypertension and to analyze MMP-9 values in these situations, correlating them with the variables obtained by ABPM. 2. To evaluate MMP-9 levels in acute BP elevations represented by hypertensive crisis. **Methods:** Participants included normotensive (NT), prehypertensive (PH) and controlled hypertensive (CHT) subjects. Individuals with hypertensive crisis, grouped as hypertensive urgency (HU) and hypertensive emergency (HE) were also included for MMP-9 measurement. Biochemical variables were evaluated for all volunteers. With the exception of hypertensive crisis, peripheral hemodynamic systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP), pulse pressure, cardiac output (CO), peripheral vascular resistance (PVR) and central variables as central systolic BP (SBPc), central diastolic BP (DBPc), augmentation index 75% (AI75%) and pulse wave velocity (PWV) were assessed by 24h

ABPM. **Results:** The PH group presented a significant difference when compared to CHT in relation to the peripheral (SBP) and central (SBPc and PWV) variables. There were also differences in these studies between the PH and NT for SBP, SBPc and DBPc. With ABPM, this study also identified significant differences in nocturnal systolic and diastolic dipping between PH and CHT. Regarding the MMP-9 levels, this study showed progressively higher levels between CHT, HU and HE, and that the PH group had higher MMP-9 levels than the CHT group. A positive correlation was identified between MMP-9 levels, CO and PVR. **Conclusions:** It is suggested that in the PH state there are changes in peripheral and central hemodynamic parameters and, consequently, in functional and structural alterations that predispose subjects to the development of systemic hypertension. Furthermore, MMP-9 levels appear to be influenced by antihypertensive therapy, since controlled hypertensive patients have lower MMP-9 levels than prehypertensive individuals. In the case of hypertensive crisis higher MMP-9 levels may constitute an important biomarker in the acute elevation of blood pressure.

Key-words: Systemic hypertension, prehypertension, metalloproteinase, arterial stiffness.

1 Introdução

1. INTRODUÇÃO

A hipertensão arterial sistêmica (HAS) é o principal fator de risco para o desenvolvimento de doenças cardiovasculares (DCV).[1,2] Por outro lado, DCV é a principal causa de mortalidade no mundo e é responsável por alta frequência de internações, ocasionando custos médicos e socioeconômicos elevados sendo, portanto, um problema de saúde global.[3,4] No Brasil, inquéritos populacionais apontam uma prevalência de HAS acima de 30%, e baixas taxas de controle, constituindo um grave problema de saúde pública. [5]

O risco para eventos cardiovasculares aumenta de forma constante a partir de 75 mmHg de pressão diastólica (PAD) usual e de 115 mmHg de pressão sistólica (PAS) usual, dobrando a cada 10 mmHg para a PAD e a cada 20 mmHg para PAS. [6] Entretanto, para o diagnóstico da HAS deve-se considerar, além da PAS ≥140 mmHg e uma PAD ≥90 mmHg em indivíduos que não estão fazendo uso de medicação anti-hipertensiva, o risco cardiovascular global, estimado pela presença de fatores de risco, a presença de lesões em órgãos-alvo (LOA) e comorbidades associadas. [7]

O termo pré-hipertensão (PH) é utilizado quando os limites de PAS variam de 120-139 mmHg ou a PAD entre 80-89 mmHg. Essa nova classificação foi introduzida em virtude da excessiva mortalidade e altas taxas de conversão para hipertensão clínica em indivíduos com elevação marginal da PA, ou seja, pré-hipertensos. [8] Suas consequências incluem, portanto, aumento do risco para o desenvolvimento da HAS,[9-11] de hipertrofia ventricular esquerda, [12,13] bem como para outros desfechos cardiovasculares e de mortalidade. [6,14,15] Dados do Strong Heart Study (SHS) apontam que nessa categoria de pressão arterial o risco de DCV é 1,8 vezes maior em comparação a normotensos, proporcionando um aumento absoluto de 6 eventos cardiovasculares para

1.000indivíduos/ano, enquanto que em pré-hipertensos diabéticos o risco é aumentado para 3,7 vezes, um aumento absoluto de 19 eventos cardiovasculares/1.000 indivíduos/ano. [16]

Dessa forma, intervenções no intuito de reduzir os níveis pressóricos, prevenir doenças cardiovasculares (DCV) e lesões em órgão-alvo, bem como evitar a progressão para hipertensão, são objetivos desejáveis a indivíduos pré-hipertensos.[17] Nesse sentido, há fortes evidências de que o tratamento com fármacos anti-hipertensivos em indivíduos pré-hipertensos reduz acentuadamente a incidência de eventos cardiovasculares recorrentes em pacientes com diagnóstico de DCV estabelecida.[18]

A eficácia da terapia anti-hipertensiva na prevenção secundária de desfechos cardiovasculares em pré-hipertensos já foi documentada em ensaio clínico, [19] entretanto, a relação custo-benefício e o risco cardiovascular a longo prazo permanecem incertos. [20] Dados apontam ainda que o risco relativo de HAS nessa população diminui em cerca de 20% com a mudança de estilo de vida , e em 34-66% com o uso de medicamentos anti-hipertensivos isolados.[21]

Outro aspecto importante a ser abordado quanto à pré-hipertensão são as alterações endoteliais presente neste grupo, tais como o aumento do tônus vasoconstritor, [22] e níveis significativamente mais elevados de angiotensina, arginina e vasopressina. [23] Outra importante alteração encontrada em pré-hipertensos inclui a redução da capacidade fibrinolítica endotelial, com consequente aumento do risco de eventos aterotrombóticos.[24] Esses achados contribuem para o aumento do risco cardiovascular em pré-hipertensos, e ressaltam a importância do diagnóstico precoce para a adoção de medidas terapêuticas otimizadas para esta população. [25]

Nesse contexto, a Monitorização Ambulatorial da Pressão Arterial (MAPA) é o método que permite o registro indireto e intermitente da PA durante 24 horas ou mais, o que permite identificar alterações do ciclo circadiano dos níveis pressóricos, bem como estabelecer estratégias terapêuticas e avaliar sua eficácia.[5,26,27]

Dentre as variáveis obtidas pela MAPA que mostram melhor correlação com os principais eventos cardiovasculares e lesões em órgãos-alvo é a pressão arterial sistólica (PAS) no sono, seguida da PAS de 24 horas e da PAS de vigília. [26,28] Outra variável que merece destaque é o descenso noturno (DN), cujo valor de normalidade é uma redução de pelo menos 10% da PA durante o sono em relação à vigília, sabe-se que existe uma correlação inversa da PA no sono e lesões em órgãos-alvo, mesmo na presença de valores normais de média de pressão obtidos pela MAPA. [29]

Atualmente, existem aparelhos de MAPA que fornecem parâmetros de avaliação da rigidez arterial, que é considerada importante fator de risco cardiovascular e comumente descrita como redução da complacência arterial.[30] Os parâmetros de rigidez obtidos pela MAPA incluem a velocidade de onda de pulso (VOP), *augmentation index* (AI – índice de incremento) e pressão arterial central, sistólica (PASc) e diastólica (PADc). [31,32] O retorno precoce das ondas refletidas da periferia para a artéria aorta ascendente durante a sístole ao invés da diástole em cada ciclo cardíaco expressa aumento da rigidez, evidenciado pela elevação da VOP, que se constitui em marcador padrão-ouro da rigidez vascular. O aumento da PASc e da pressão de pulso (PP), que são preditores independentes de DCV, ocorrem como consequência do retorno precoce dessas ondas e podem refletir o grau de rigidez arterial. [33,34]

Estudos sugerem que a rigidez arterial estaria associada a níveis elevados de um grupo de enzimas proteolíticas denominadas metaloproteinase-9, não somente em

portadores de hipertensão sistólica isolada, mas também em indivíduos jovens sem comorbidades. [35,36] A MMP-9 emergiu recentemente como preditor de risco em pacientes com DCV [37], tendo sido sugerido que o aumento de sua expressão possa prejudicar o relaxamento vascular e, consequentemente, contribuir para a hipertrofia na parede das artérias, causando disfunção vascular e contribuindo para o aumento da rigidez arterial [38].

Na hipertensão arterial ocorrem mudanças na parede arterial conhecidas como remodelamento vascular [39]. Um mecanismo pelo qual o remodelamento vascular ocorre é por meio da degradação e reorganização da matriz da parede do vaso, com participação de MMPs [40, 41]. Além disso, a ativação do sistema renina-angiotensina induz efeitos na estrutura vascular, como crescimento e fibrose, sendo um importante regulador do remodelamento e inflamação vascular [42].

As MMPs degradam várias proteínas da matriz extracelular quebrando-as em suas ligações peptídicas específicas e são expressas em vários tipos celulares e tecidos, incluindo células da musculatura vascular lisa, endotélio, fibroblastos e células inflamatórias [40, 43, 44].

A atividade das MMPs pode ser regulada por diversos mecanismos, entre eles a indução da transcrição gênica, modificação pós-traducional e interação das MMPs com seus inibidores teciduais endógenos, os TIMPs (inibidores teciduais de MMPs) [45-47]. Em condições fisiológicas, existe um equilíbrio entre a razão de MMPs e TIMPs. Entretanto, em processos patológicos como a hipertensão arterial, ocorre um desequilíbrio dessa razão, levando à degradação excessiva das proteínas da matriz extracelular e, consequentemente, ao remodelamento vascular patológico [47,48].

Assim, em pacientes hipertensos, níveis elevados de MMP-9 podem levar à degradação da elastina, enquanto a redução de seu inibidor endógeno TIMP-1, pode levar ao acúmulo de produtos de degradação de fibrina, resultando em deposição inadequada de colágeno [38].

Apesar de relatos dessa associação, a literatura carece de estudos envolvendo dosagens de MMPs e sua associação com marcadores de rigidez arterial em populações com diferentes níveis de pressão arterial. Dessa forma, a realização deste estudo objetivou avaliar parâmetros hemodinâmicos periféricos e centrais, obtidos pela MAPA, e sua correlação com níveis de MMP-9.

O presente estudo também objetivou avaliar a relação entre MMP-9 e diferentes níveis de PA em casuística de normotensos, pré-hipertensos e indivíduos com crise hipertensiva.

2 Artigos Científicos

CIRCULATING LEVELS OF MATRIX METALLOPROTEINASE-9 ARE ELEVATED IN INDIVIDUALS WITH HYPERTENSIVE CRISIS

Short Title: Metalloproteinase-9 in Hypertensive Crisis

Abstract

Background: Matrix metalloproteinase-9 (MMP-9) participates in the degradation of components of the extracellular matrix and it is involved on vascular remodeling. The imbalance between their activation and inactivation mechanisms seems to be associated with vasomotor changes. The aim of this study was to investigate the plasma levels of MMP-9 in acute vascular alterations due to hypertensive crisis. Methods: Forty normotensive (NT) and 58 controlled hypertensive subjects (CHyp) participated in this study as well as 57 patients in hypertensive emergencies (HypEmerg) and 43 in hypertensive urgencies (HypUrg). Results: The mean ages were 43.5, 57.7, 59.4 and 62.4 years for the NT, CHyp, HypUrg and HypEmerg groups, respectively. The mean blood pressures were 116.5±13.9/72.4±10.6 mmHg for NT, 123.2±12.6/79±9.2 for CHyp, 194.1±24.3/121.4±17.3 for HypUrg and 191.6±34.3/121.7±18.8 mmHg for HypEmerg, respectively (p-value<0.0001 between groups). MMP-9 levels were statistically different between the HypEmerg (2.31±0.2 ng/mL) and HypUrg groups (2.17±0.3 ng/mL) compared to the NT (1.94±0.3 ng/mL) (*p-value*<0.01 and *p-value*<0.05, respectively) and CHyp groups (1.92±0.2 ng/mL) (*p-value*<0.01). There was no difference in the MMP-9 levels between the different clinical presentations of hypertensive crisis.Conclusions: Matrix metalloproteinase-9 concentrations are progressively higher in the order normotensive, controlled hypertensive, hypertensive urgency and emergency

groups with significant differences between the hypertensive crisis groups (urgency and emergency) compared to the other groups. Therefore, MMP-9 may be a new biomarker in cases of acute elevations of blood pressure.

Keywords: Matrix metalloproteinase, cardiac biomarkers, hypertension, emergency, cardiovascular pathophysiology

Introduction

Systemic arterial hypertension (SAH) is an important public health problem and a modifiable risk factor closely associated to cardiovascular diseases (CVD), the leading cause of death worldwide [1]. In this context, clinical trials have shown that the reduction in blood pressure (BP) levels results in a reduction of the risk to develop strokes, coronary artery disease, heart failure and renal insufficiency [2].

Vascular remodeling, an adaptive response that attempts to restore the vascular mechanical balance and the BP, is one of several pathophysiological mechanisms that characterize the multifactorial etiology of hypertension [3,4]. However, pathological remodeling is associated with vascular changes including endothelial dysfunction, smooth muscle cell hypertrophy of the arteries, and cell migration and proliferation with resulting thickening of the vascular wall and structural changes of the extracellular matrix (ECM) [5,6].

Degradation and reorganization of the vascular wall matrix results from the activation of proteolytic pathways such as matrix metalloproteinases, especially gelatinase (MMP-2 and MMP-9), a group of zinc-dependent endopeptidases

whose imbalance between activation and inhibition results in excessive degradation of ECM proteins [6-8].

Recently, high levels and activity of MMP-2 and MMP-9 have been demonstrated in hypertensive patients [9,10] and in animal models [5,7,11,12]. The levels of MMP-9 has been associated with cardiovascular diseases due to its proteolytic activity on type IV collagen, one of the main constituents of the basal membrane that involves vascular smooth muscle cells and the endothelium, exerting an important role in cell migration and infiltration in the atherosclerotic process [13]. In addition, the degradation of elastin by MMP-9 is implicated in the process of arterial stiffness and the development of aneurysms [8,10,14].

In view of the scarcity of studies evaluating MMP-9 levels at different BPs and especially with acute alterations of BP, the objective of the present study was to investigate MMP-9 as a biomarker of acute vascular alterations resulting from acute BP elevations characterized as hypertensive crisis.

Methods

The Research Ethics Committee of the institution approved the study protocol according to national and international guidelines (CAAE no. 07606212.5.0000.5415, no. 94.248/2012). Subsequently, the individuals were informed about the objectives of this study and consulted about their interest and consent to participate as volunteers, in a way that, regardless of their choice, their treatment would not suffer. The current study was performed according to the ethical standards of the Helsinki Declaration.

The control group (NT) consisted of 40 normotensive patients who had systolic blood pressure (SBP) <140 mmHg and diastolic blood pressure (DBP) <90 mmHg without taking antihypertensive drugs. A controlled hypertensive group (CHyp) was comprised of 58 subjects with SBP <140 mmHg and DBP <90 mmHg while taking antihypertensive drugs, who were being followed up in a university service specialized in hypertension and agreed to participate in the study.

A total of 100 individuals aged ≥ 18 years presenting hypertensive crisis and admitted to the Clinical Emergency Department of the university hospital were evaluated in the third group. They were grouped as hypertensive emergency (n = 57) and urgency patients (n = 43). The hypertensive emergency group (HypEmerg) was characterized by subjects with elevated levels of SBP ≥ 180 mmHg and/or DBP ≥ 120 mmHg complicated by evidence of progressive acute target organ damage (TOD), such as hypertensive encephalopathy, hemorrhagic or ischemic stroke, acute myocardial infarction, left ventricular failure with acute pulmonary edema, unstable angina pectoris, acute aortic dissection or acute and progressive renal failure. On the other hand, hypertensive urgencies (HypUrg) were defined as increases in BP without TOD [1,15,16].

The exclusion criteria adopted included low life expectancy, previous diagnosis of hypertension or prior use of antihypertensive drugs (valid for NT participants), chronic diseases that could limit participation in the study (e.g. tumors), difficulty in understanding, inability to measure BP, and refusal to sign the informed consent form. Additionally, female patients presenting with preeclampsia and eclampsia, and hypertensive patients with pseudocrisis were

excluded. Emergency Department BP was measured thrice according to VII National Joint Committee guidelines [16], using an automatic digital blood pressure monitor (OMRON Healthcare Inc., Bannockburn, IL, USA). The recorded BP was the mean of the three readings.

An investigative protocol was used to collect information on patient history, associated diseases (diabetes mellitus), medications, smoking, and family history. The weight and height were measured using anthropometric scales. Peripheral blood was collected to measure serum glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), creatinine, potassium, and uric acid levels. The measurement of glycemia, CT, HDL-c and TG were by the enzymatic colorimetric method and the LDL-c fraction was calculated for TG <400 mg/dL using the formula LDL-c = CT-HDL-c - TG / 5. The creatinine concentration was calculated using a kinetic colorimetric assay and serum potassium was assessed by selective electrode and ion tests. The glomerular filtration rate (GFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration - CKD-EPI creatinine equation [17].

Venous blood samples were also collected in EDTA vacutainer tubes (Becton-Dickinson, São Paulo, Brazil) by venipuncture, and centrifuged at 3500 rpm for 10 minutes with plasma fractions being immediately stored at -70°C until measurement of the MMP-9.

The levels of MMP-9 were assessed using the Human Matrix metalloproteinase-9 Quantikine ELISA kit (R & D Systems, Inc., Minneapolis, MN, USA) with a calculation of medians being presented as nanograms per milliliter (ng/mL). Subsequently, MMP-9 values were transformed into logarithms to reflect

normal distribution for statistical analysis. Data are presented as means ± standard deviation (SD) for continuous variables and as percentages (%) for categorical variables. The comparison between the groups in relation to the continuous variables was performed by analysis of variance (ANOVA) for parametric and Kruskal-Wallis for non-parametric test. Qualitative variables were analyzed using the chi-square or Fisher's exact test. Univariate and multivariate regression analysis was performed to identify the influence of independent variables on MMP-9 levels, with p-values <0.05 being considered significant.

Results

The study included individuals aged between 22 and 92 years old with a mean age of 43.5 years in the NT group (11 men); 57.7 years in the CHyp group (29 men); 59.4 years in the HypUrg group (21 men) and 62.4 years (31 men) in the HypEmerg group. Table 1 shows the characteristics of the population of the present study including BP levels and medications taken, and Table 2 shows the levels of the analyzed biochemical variables.

There were significant differences in MMP-9 levels between groups (p-value <0.0001). The HypEmerg group (2.31 ± 0.29 ng/mL) had a significantly higher level of MMP-9 compared to the NT (1.94 ± 0.32 ng/mL; p-value <0.01) and CHyp groups (1.92 ± 0.23 ng/mL; p-value <0.01). There were also significant differences between the HypUrg group (2.17 ± 0.3 ng/mL) and the NT (p-value <0.05) and CHyp groups (p-value <0.01). Although the MMP-9 level was higher in the HypEmerg compared to the HypUrg group, no statistically significant difference was found.

The descriptive and ANOVA statistical analyses of the expressions of MMP-9 are presented in Table (online supplemental material), and plotted as absolute values in Figure 1. The correlation coefficients of MMP-9 with clinical-biochemical variables are showed in Table 3 and the results of multiple regression are presented in Table 4.

Figure 2 shows characteristics related to age, gender and absolute values of MMP-9 in the HypEmerg group. There was no difference in MMP-9 levels between the different clinical presentations of hypertension emergencies (acute pulmonary edema, stroke, myocardial infarction, unstable angina and hypertensive encephalopathy; P-value = 0.9).

Discussion

Matrix metalloproteinases-9 has been associated with several structural and functional changes of the cardiovascular system and, consequently, the development and progression of cardiovascular diseases [13,14,18-20]. In this study, MMP-9 expression was evaluated in hypertensive crisis, with levels that were progressively higher in the HypUrg and HypEmerg groups, suggesting that when inflammatory mechanisms are present [21,22], the levels of MMP-9 may be associated with increased cardiovascular risk. In this context, these results also suggest that MMP-9 levels could constitute an important biomarker of cardiovascular risk, as well as of the endothelial changes present in SAH.

These data corroborate several clinical and experimental studies that described the association of MMP-9 levels with the incidence of acute cardiovascular disease and chronic hypertension [8-10, 13,14, 18-20]. Other studies also

suggest that the increase of MMP-9 in healthy individuals may predispose them to cardiovascular diseases, because high levels of MMP-9 have been observed in acute cardiovascular events in individuals without known clinical diseases [19,20].

In the current study, there was no significant difference in MMP-9 levels between the NT and CHyp groups, possibly due to the use of antihypertensive drugs by the hypertensive population. These data are corroborated by the finding of higher levels of MMP-9 in untreated hypertensive rats [23], as well as the reduction of BP levels and prevention of thickening of the tunica media in hypertensive animal models treated with MMP-9 inhibitors. In addition, MMP-9 levels were reduced in clinical trials evaluating the effect of different antihypertensive drugs, such as calcium channel blockers (lercadipine), while for others, such as felodipine, diltiazem and an angiotensin-converting enzyme inhibitor (enalapril), MMP-9 levels did not change, suggesting that the discrepancies in the results of different studies may result from drug therapy [5, 24-27].

The reduction of MMP-9 levels due to the use of antihypertensive drugs was also observed in a study by Onal et al., who evaluated levels of MMP-9 in 33 hypertensive stage 1 patients, pre and post treatment with antihypertensive medications (Candesartan 8 mg/day prescribed to 17 patients and lisinopril 10 mg/day given to 16 patients). The authors observed that after antihypertensive treatment there was a reduction in MMP-9 levels, as well as an elevation in the levels of tissue inhibitors of matrix metalloproteinases-1 (TIMP-1), an endogenous regulator of MMP-9 activity, suggesting that drug therapy may influence the regulation of activation-inhibition of matrix metalloproteinases [24].

The authors observed that after antihypertensive treatment there was a reduction in MMP-9 levels, as well as an elevation in the levels of TIMP-1, an endogenous regulator of MMP-9 activity, suggesting that drug therapy might influence the activation-inhibition regulation of matrix metalloproteinases [24].

Similar data were described in a sub-study of the ASCOT study, in which the MMP-9 levels was evaluated in 96 hypertensive patients before and after antihypertensive treatment. The authors concluded that the increases in MMP-9 and TIMP-1 compared to normotensive patients at baseline could reflect an increase in the deposition and retention of type I collagen in the extracellular cardiac and vascular matrix. In addition, the authors suggested an association between elevated levels of MMP-9 and increased cardiovascular risk calculated using the Framingham risk score, as well as a possible role of this marker in the prognosis of cardiovascular events in hypertensive patients [28].

In an experimental study on the other hand, spontaneously hypertensive rats were treated with high doses of Xuezhikang, a red rice yeast extract that contains natural lovastatin and its counterparts. This treatment resulted in reduced MMP-9 levels and increased nitric oxide production [29]. This rice yeast extract is widely used in Chinese medicine to treat patients with cardiovascular diseases.

In this context, it is clear that there is evidence that MMP-9 plays an important role in structural alterations associated with hypertension and its complications [23]. However, there are controversies regarding the expression of MMP-9 and its endogenous inhibitor (TIMP-1) reported in other studies indicating unchanged [9], higher [10, 23-25, 30,31] and even lower MMP levels [32,33] to those of

normotensive individuals. So, the results of the present study suggest the importance of considering the different drug classes used when assessing MMP-9 plasma levels. In this sense, some of the controversy found in the literature may be due to different inclusion/exclusion criteria for participants selection, the severity of hypertension and the presence of comorbidities among others as causal hypotheses [26]. These variables point to a need for further studies to verify the modulation between activation and inhibition of MMP-9, as its elucidation and control may constitute a diagnostic marker as well as therapeutic agent.

The only independent explanatory variable for MMP-9 levels was uric acid (p-value = 0.002), as corroborated by studies that point to uric acid as an independent predictor for the development of hypertension in view of its reductive effect on vasodilatory response [34-36]. However, considering that the level of uric acid depends on metabolic factors linked to enzymatic activity, among other things, higher levels of uric acid could contribute to an increase of MMP-9 activity. In addition, the association between elevated serum uric acid levels and markers of arterial stiffness, such as pulse wave velocity and the augmentation index [37,38], corroborate our findings, in view of the action of MMP-9 in the degradation and reorganization of the vascular wall, and its consequent implication in the process of arterial stiffness [6,7,10,14]. Moreover, uric acid contributes to systemic inflammation in humans and hypertension is an inflammatory disease [39,40]. Thus, in other words, acute elevation of BP represents an inflammatory state more accentuated.

We cannot fail to highlight the strength of the findings of this study that compared the MMP-9 levels in hypertensive crisis to normotensive and controlled

hypertensive subjects, and also between different presentations of hypertensive emergencies, which has not been evaluated by other studies. Progressively higher levels of MMP-9 were associated with higher BP levels present in cases of acute increases in pressure, defined as hypertensive crisis. However, some limitations need to be mentioned. In the study groups, the expression of TIMP-1, an inhibitor of MMP-9, was not evaluated, which could contribute to a better understanding of the relationship between MMP-9/TIMP-1 in hypertensive crisis. Another limiting factor to be considered is related to the analysis of MMP-9 levels that were restricted to a single moment (hypertensive crisis), while a post-crisis evaluation could provide information on the activation and inhibition of MMP-9 after the event.

To the best of our knowledge, this is the first study that evaluates matrix metalloproteinase levels in hypertensive crisis, with MMP-9 levels being progressively higher in the normotensive, controlled hypertensive, and hypertensive urgency and emergency groups. Statistically significant differences were observed between the hypertensive crisis groups compared to the other groups, indicating that when inflammatory mechanisms are present, MMP-9 appears to be activated, thereby increasing cardiovascular risk. In this context, new studies are needed to verify the modulation between the activation and inhibition of MMP-9, since its elucidation and control may constitute a diagnostic marker as well as a potential therapeutic agent.

Disclosure

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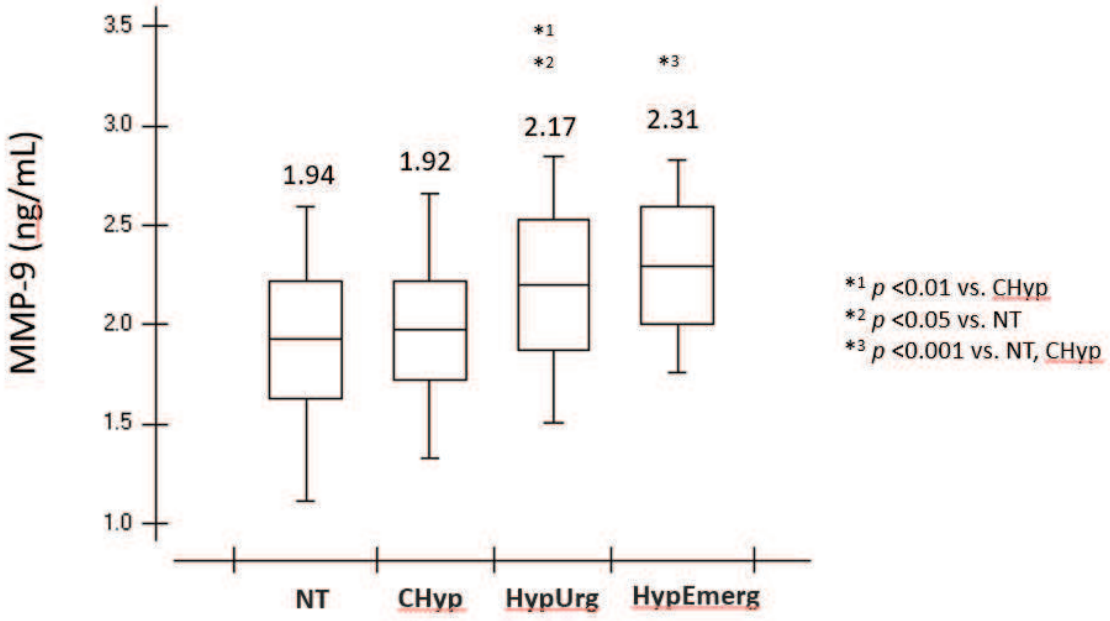
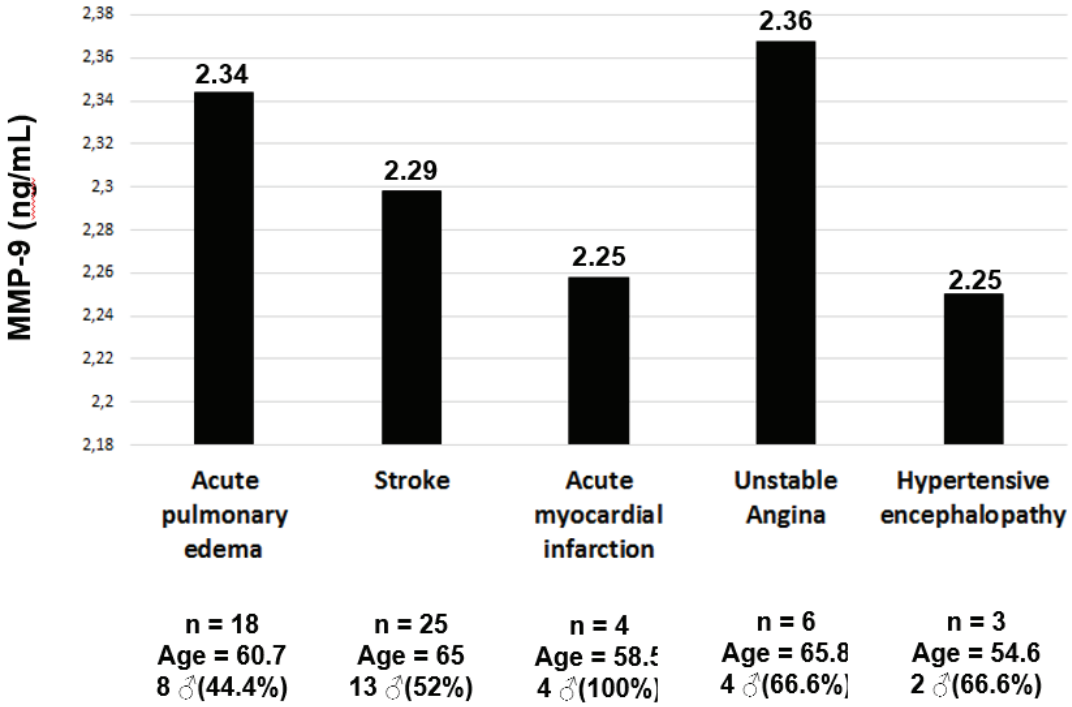
Figure 1 - Circulating metalloproteinase-9 levels in the four studied groups.



The values are presented as log (ng/mL). NT = normotensive group; CHyp = controlled hypertensive group; HypUrg = hypertensive urgency; HypEmerg = hypertensive emergency

Figure 2 - Circulating metalloproteinase-9 levels in several clinical presentations of hypertensive emergency.



The values are presented as log (ng/mL).

Table 1. Clinical characteristics of hypertensive patients and normotensive subjects and those in hypertensive crisis.

Variable	NT (*n*=40)[a]	CHyp (*n*=58)[b]	HypUrg (*n*=43)[c]	HypEmerg (*n*=57)[d]	*p*-value *(axbxcxd)*	a x b	a x c	a x d	b x c	b x d	c x d
Age (years)	43.5 ± 10.2	57.7 ± 7.4	59.4 ± 15.6	62.4 ± 14.3	<0.0001#	<0.01*	<0.01*	<0.01*	NS	NS	NS
Gender (male; %)	11;27.5%	29;50%	21;48.9%	31;54.4%		0.03*	NS	0.01*	NS	NS	NS
Skin color (White; %)	33 (82.5)	50 (86.2)	27 (62.8)	44 (77.2)		NS	NS	NS	0.009*	NS	NS
BMI (kg/m^2)	23.7 ± 3.3	29.6 ± 3.4	30.1 ± 7.2	27.6 ± 6.3	0.002*	<0.01*	<0.01*	0.006	NS	0.01	0.03
Smokers (%)	6 (15%)	11 (19)	7 (16.3)	17 (29.8)		NS	NS	NS	NS	NS	NS
History of diabetes (%)	-	9 (15.5)	12 (27.9)	23 (40.3)		0.009*	0.0002#	0.0000#	NS	0.003*	NS
Blood Pressure											
SBP (mmHg)	116.3 ± 11.8	123.2 ± 10.5	194.1 ± 28.6	191.6 ± 34.3	<0.0001#	NS	<0.01*	<0.01*	<0.01*	<0.01*	NS
DBP (mmHg)	72.3 ± 9	79 ± 7.7	121.4 ± 17.3	121.7 ± 18.8	<0.0001#	NS	<0.01*	<0.01*	<0.01*	<0.01*	NS
***Drugs, n* (%)**											
Antidiabetic drugs (%)	-	16 (27.6)	4 (9.3)	13 (22.8)		-	-	-	0.02*	NS	NS
Statins (%)	-	14 (24.1)	15 (34.9)	22 (38.6)		-	-	-	NS	NS	NS
Diuretics (%)	-	48 (82.8)	26 (60.4)	29 (50.9)		-	-	-	0.02*	0.0003#	NS
ARB (%)	-	25 (43.1)	16 (37.2)	18 (28)		-	-	-	NS	NS	NS
ACEi (%)	-	22 (38)	18 (41.8)	31 (45.6)		-	-	-	NS	NS	NS
CCB (%)	-	18 (31)	14 (32.6)	18 (31.6)		-	-	-	NS	NS	NS
B-blockers (%)	-	16 (27.6)	26 (60.4)	27 (47.4)		-	-	-	0.001#	0.03*	NS
Antiaggregant and/or anticoagulant (%)	-	19 (32.8)	19 (44.2)	37 (64.9)		-	-	-	NS	0.0008#	0.04*

NT = normotensive group; CHyp = controlled hypertensive group; HypUrg = hypertensive urgency; HypEmerg = hypertensive emergency; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; ARB = angiotensin receptor blocker; ACEi = angiotensin converting enzyme inhibitor; CCB = calcium channel blockers. **p-value <0.05; #p-value <0.001*; NS - non-significant.

Table 2. Biochemical parameters of hypertensive patients and normotensive subjects and those in hypertensive crisis

Variable	NT (*n*=40)[a]	CHyp (*n*=58)[b]	HypUrg (*n*=43)[c]	HypEmerg (*n*=57)[d]	*p* value *(axbxcxd)*	a x b	a x c	a x d	b x c	b x d	c x d
Biochemical parameters											
glycemia (mg/dL)	86.6 ± 16.7	104.8 ± 42.6	121.5 ± 45.5	149.7 ± 96.3	<0.0001#	<0.01*	<0.01*	<0.01*	<0.05*	<0.05*	NS
HDL-c (mg/dL)	61.2 ± 12.2	53 ± 13.2	52.6 ± 19.8	48.1 ± 16.4	0.008*	0.02	0.004	<0.01*	NS	NS	NS
LDL-c (mg/dL)	115.2 ± 23.3	123.6 ± 31.8	127.1 ± 38.5	109.3 ± 44.8	0.03*	NS	NS	NS	NS	0.03	0.01
Cholesterol (mg/dL)	198.6 ± 22.1	204.3 ± 37.4	210.7 ± 49	184.6 ± 52.4	0.009*	NS	NS	NS	NS	NS	<0.05*
Triglycerides (mg/dL)	110.4 ± 48.1	137.2 ± 59.1	142.7 ± 69.3	130.1.1 ± 77.7	NS	-	-	-	-	-	-
Creatinine (mg/dL)	0.85 ± 0.21	0.86 ± 0.18	1.55 ± 1.65	1.83 ± 1.65	0.001#	NS	0.03	<0.01*	<0.05*	<0.01*	NS
Urinary Sodium	159.5 ± 46.3	146.1 ± 52.2	128 ± 50.4	120.4 ± 43.9	<0.0001#	NS	0.002*	<0.01*	NS	0.005*	NS
GFR (mL/min/1.73m²)	94.4 ± 17.7	87.7 ± 16.5	70.8 ± 36.1	59.9 ± 34.5	<0.0001#	NS	0.002*	<0.001*	<0.05*	<0.001#	NS
Uric acid (mg/dl)	3.8 ± 1.01	5.7 ± 1.43	6.5 ± 2.17	6.2 ± 2.15	<0.0001#	<0.01*	<0.01*	<0.01*	NS	NS	NS
Potassium (mEq/L)	4.25 ± 0.37	4.34 ± 0.57	4.55 ± 0.87	4.47 ± 0.83	NS	-	-	-	-	-	-
LogMMP-9	1.94 ± 0.3	1.92 ± 0.2	2.17 ± 0.3	2.31 ± 0.2	0.000#	NS	0.05	<0.001#	0.001	<0.001#	NS

Values are means±SD. NT = normotensive group; CHyp = controlled hypertensive group; HypUrg = hypertensive urgency; HypEmerg = hypertensive emergency; HDL-c = high-density lipoprotein cholesterol; LDL-c = low-density lipoprotein; GFR = glomerular filtration rate.

**p-value <0.05; #p-value <0.001*; NS - non-significant.

Table 3 - Correlation coefficients of MMP-9 with clinical-biochemical variables.

Variable	**r**	**P-value**
Age (years)	0.105	NS
Gender (male; %)	0.103	NS
Smokers (%)	0.072	NS
History of diabetes (%)	0.167	0.024*
BMI (kg/m^2)	0.049	NS
SBP (mmHg)	0.381	0.000#
DBP (mmHg)	0.387	0.000#
Fasting glycemia (mg/dL)	0.164	0.028*
HDL-c (mg/dL)	-0.070	NS
LDL-c (mg/dL)	-0.032	NS
Total cholesterol (mg/dL)	-0.036	NS
Triglycerides (mg/dL)	0.047	NS
Serum creatinine (mg/dL)	0.234	0.002*
GFR (mL/min/1.73m²)	-0.240	0.001*
Uric acid (mg/dL)	0.327	0.000#
Potassium (mEq/L)	0.100	NS

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL-c = high-density lipoprotein cholesterol; LDL-c = low-density lipoprotein; GFR = glomerular filtration rate. **p-value <0.05; #p-value <0.001*; NS - non-significant.

Table 4 - Multivariate regression for metalloproteinase-9 levels.

Variable	**β**	**SEβ**	**P-value**
History of diabetes (%)	0.027	0.034	NS
SBP (mmHg)	0.001	0.112	NS
DBP (mmHg)	0.004	0.362	0.000#
Fasting glucose (mg/dL)	0.000	0.069	NS
Serum creatinine (mg/dL)	-0.015	-0.058	NS
GFR (mL/min/1.73m²)	0.000	-0.022	NS
Uric acid (mg/dL)	0.033	0.200	0.003*

SBP = systolic blood pressure; DBP = diastolic blood pressure; GFR = glomerular filtration rate. **p-value <0.05; #p-value <0.001*; NS - non significant

ARTERIAL STIFFNESS PARAMETERS ARE ALTERED IN PREHYPERTENSIVE INDIVIDUALS

Short Title: **Arterial Stiffness in Prehypertension**

Abstract

Introduction: Prehypertension predisposes subjects to arterial hypertension and increased cardiovascular morbidity and mortality. Furthermore, central blood pressure and the arterial stiffness index have been associated with higher cardiovascular mortality. Thus, the present study assessed biochemical variables, and peripheral and central hemodynamics (markers of arterial stiffness) in prehypertensive subjects. **Methods:** We compared the results of clinical and biochemical evaluations and ambulatory blood pressure monitoring (ABPM) of 47 normotensive (NT), 39 prehypertensive (PH), and 138 controlled hypertensive subjects (CHT). We evaluate peripheral [systolic blood pressure (SBP) and diastolic BP (DBP)] and central hemodynamic [central SBP (cSBP), cDBP, augmentation index (AI75%) and wave velocity pulse (PWV)] parameters using ABPM. Central hemodynamic parameters were determined by brachial oscillometry using the Mobil-O-Graph® system. **Results:** The mean ages of the NT, PH and CHT were 48.3 ± 10.6, 50.1 ± 9.6 and 57.7 ± 10.9 years, respectively (p-value = 0.000). Metabolic parameters (glycemia, total cholesterol, LDL-cholesterol and triglycerides) were statistically different in PH compared to CHT, while peripheral and central hemodynamic parameters were higher in the CHT group. There were significant differences between the groups in the three ABPM periods (24-h, wake and sleep) for the peripheral (SBP, DBP, mean arterial pressure, total vascular resistance) and central (cSBP, cDBP, AI75% and PWV) parameters. **Conclusion:** Prehypertensive individuals had higher peripheral and

central blood pressures compared to normotensive subjects but lower than controlled hypertensive patients in the three periods (24-h, wake and sleep). These results suggest that in the prehypertensive group there are already functional and structural alterations that predispose individuals to the development of hypertension.

Keywords: Blood Pressure; Prehypertension; Central blood pressure; Arterial stiffness.

Introduction

Prehypertension is considered a forerunner of arterial hypertension (AH) and is associated with increased morbidity and mortality due to cardiovascular diseases (CVD), which account for about 32% of deaths in Brazil [1-3]. Even with few epidemiological studies, the prehypertensive blood pressure (BP) levels determined by the VII Joint National Committee (systolic BP of 120-139 mmHg and diastolic BP of 80-89 mmHg) categorized many individuals, previously considered normotensive, as prehypertensive; the prevalence is about 38% [4-6].

Increased risk for CVD, which starts at BPs of 115/75 mmHg, doubles with each increment of 20 mmHg of systolic pressure and 10 mmHg of diastolic pressure [1,4,7]. Thus, prehypertension has become the most common risk factor for the development of AH and is considered a high risk factor for target organ lesions such as myocardial infarction and coronary atherosclerotic disease, with consequent Increased mortality [5,8-10]. Data from the Strong Heart Study (SHS) indicate that in this category of BP, the risk of CVD is 1.8 times higher than for normotensive patients, causing an absolute increase of six cardiovascular events/1000 individuals/year [11].

In addition to BP, functional and morphological parameters have been described as significantly higher in prehypertension compared to normotension, including heart rate (HR), pulse pressure (PP), mean arterial pressure (MAP), end systolic stress and end isovolumetric systolic stress [12]. In addition, other parameters such as central pressure, augmentation index (AI75%) and pulse wave velocity (PWV), parameters used as markers of arterial stiffness, have been associated with increased cardiovascular mortality, besides being identified as better predictors of CVD [8,13,14]. Furthermore, studies indicate that arterial stiffness is correlated with age, triglyceride levels, SBP, 24-h PP, urinary albumin

excretion and carotid artery intima-media thickness, factors that are prevalent in the prehypertensive population of which 64% of under 60-year-old and 94% of over 60-year-old individuals present one or more risk factors for CVD [6,7,15].

Thus, early diagnosis and the establishment of therapeutic measures in prehypertensive patients aim to reduce the risk of hypertension, CVD and death [12,16,17]. Although the diagnosis of AH is based on the detection of high and sustained BP levels in the doctor's office, ambulatory blood pressure monitoring (ABPM) allows the indirect and continuous recording of BP for 24 hours or more. Hence, it is possible to identify changes in the BP circadian cycle, establish therapeutic and prognostic strategies, and evaluate the efficacy of antihypertensive therapy [18,19].

Sleep SBP, 24-h SBP and wakefulness SBP are among the variables obtained by ABPM that are most correlated with cardiovascular events and lesions in target organs [19,20]. However, another variable that deserves attention is nocturnal dipping, which shows an inverse relationship with target organ lesions [21-23]. Thus, the objective of the present study was to evaluate peripheral (BP, HR and PP) and central (cSBP, cDBP, AI75% and PWV) hemodynamic parameters in prehypertensive individuals compared to normotensive and hypertensive controls.

Methods

Subjects

The present study was performed at the Hypertension Outpatient Clinic of the Medical School after approval by the Research Ethics Committee, and authorization of the participants through their written informed consent.

A cross-sectional study enrolled 224 individuals aged 23-79 years, of whom 47 were normotensive, 39 were prehypertensive and 138 were controlled hypertensive

patients. Normotension was characterized by systolic blood pressure (SBP) <120 mmHg and diastolic blood pressure (DBP) <80 mmHg without the use of antihypertensive agents. Prehypertension was defined by office BP values between 120 and 139 mmHg for SBP and/or between 80 and 89 mmHg for DBP. In an outpatient clinic follow-up, controlled hypertension was defined as SBP <140 mmHg and DBP <90 mmHg with the patient taking antihypertensive drugs.

Exclusion criteria included pregnancy, low life expectancy, previous diagnosis of AH or previous use of antihypertensive drugs [valid for the normotensive (NT) and prehypertensive (PH) groups], impaired understanding of participation in the study and impossibility to measure arterial BP.

Clinical and biochemical analysis

Data on age, gender, weight, height, body mass index [BMI = weight (kg)/height squared (cm^2)], medications, presence of comorbidities and diabetes mellitus were obtained by interview and verified in the patient's medical records. Patients were defined as diabetics when they had been previously treated with hypoglycemic agents or had fasting glucose levels ≥ 126 mg/dL on at least two different occasions.

For biochemical analysis, peripheral blood was collected during fasting. The biochemical parameters investigated were uric acid, serum creatinine by kinetic colorimetric assay, and potassium by selective electrode and ion tests [24].

Glycemia, total cholesterol, high-density lipoprotein cholesterol (HDL-c) and triglycerides were measured by enzymatic colorimetric methods [25]. The low-density lipoprotein cholesterol (LDL-c) fraction was calculated using the Friedewald formula [26]. The estimated glomerular filtration rate (eGFR) was calculated using the MDRD (Modification of Diet in Renal Disease) formula [27] and Cockroft-Gault equation[28].

Ambulatory blood pressure monitoring

All participants were submitted to ABPM, performed on a day of standard activity, with a cuff adequate for the individual's arm size, using the Mobil-O-Graph® 24h PWA Monitor [29,30]. The parameters obtained by the device included the SBP, DBP, MAP, HR, PP, cSBP, cDBP, Al75%, total vascular resistance (PVR) and PWV. All variables were measured at 30-minute intervals with the mean being obtained for the three periods (24-h, wakefulness and sleep).

Nocturnal dipping was standardized as ≥ 10% drop in SBP and DBP from wakefulness to sleep. Inverted dipping as defined when the drop was < 0%, no decrease when the drop was < 10%, and maximum value ≥ 20%, according to the validated protocol of the British Hypertension Society. The Brazilian Guidelines were used to carry out the ABPM [31,32].

Statistical analysis

Descriptive analysis was used for qualitative variables with the data being presented as means ± standard deviation. A p-value <0.05 was considered significant. The distribution of all variables was assessed using the Shapiro-Wilk normality test. ANOVA was used to compare normal quantitative variables, Kruskal-Wallis for non-normal quantitative variables and Fischer's exact test was used to compare the qualitative variables related to the characteristics of the participants. All statistical analyzes were performed using SPSS version 24.0 (SPSS Inc., Chicago, Ill., USA).

Results

A total of 224 subjects (104 male - 46.4%) were studied - 47 normotensive (9 male - 19.1%), 39 prehypertensive (34 male – 85.0%) and 138 controlled hypertensive (61 male

- 43.5%). The gender, mean age, BMI, and biochemical variables, as well as comparisons between groups are presented in Table 1.

There were significant differences in the PH group compared to the NT and controlled hypertensive (CHT) groups regarding gender (p-value = 0.000 and 0.000, respectively) and triglyceride levels (p-value = 0.000 and 0.02, respectively). The PH group also presented statistical differences in relation to the NT group in respect to creatinine levels (p-value = 0.000), uric acid (p-value = 0.000) and HDL-c (p-value = 0.000). Compared to the CHT group, the PH group was statistically different in relation to age (p-value = 0.000), history of diabetes (p-value = 0.000), glucose levels (p-value = 0.000), eGFR (p-value = 0.01) in both models (MDRD and Cockroft-Gault), total cholesterol (p-value = 0.000), and LDL-c (p-value = 0.002). As there was no difference between the MDRD and Cockroft-Gault calculations, we will present only the MDRD results.

There were no significant differences between the groups regarding race, smoking and serum potassium levels. Although there was no statistical difference in albuminuria, levels increased between groups.

In the comparison of the variables obtained by ABPM (Table 2), over 24 hours, the PH group had significantly higher mean levels compared to the NT group for SBP (p-value = 0.000), cSBP (p-value = 0.000), MAP (p-value = 0.000), cSBP (p-value = 0.000) and cDBP (p-value = 0.002). In relation to the AI75%, the PH group also differed from the NT group (p-value = 0.008), but with a lower mean value. In the wakefulness period, the results between the PH and NT groups were significantly different in respect to the same variables as the 24-h period [SBP (p-value = 0.000), DBP (p-value = 0.000), MAP

(p-value = 0.000), SBP (p-value = 0.000), cDBP (p-value = 0.000) and AI75% (p-value = 0.006)] as well as an increased cardiac output (p-value = 0.006).

Over 24 hours, the PH Group presented significantly lower values for the SBP (p-value = 0.02), PP (p-value = 0.02), cSBP (p-value = 0.01), AI75% (p-value = 0.001) and PWV (p-value = 0.000). In the wakefulness period, the results of the PH group were significantly lower compared to the CHT group for the PP (p-value = 0.03), AI75% (p-value = 0.004) and PWV (p-value = 0.000).

During the sleep period, there were statistically significant differences between the PH group and both the NT and CHT groups for the SBP (p-value = 0.01; p-value = 0.000, respectively) and MAP (p-value = 0.01; p-value = 0.02, respectively), with the values being progressively higher from the NT group to the PH, to the CHT group. In relation to the NT group, the DBP (p-value = 0.000) and cDBP (p-value = 0.000) were significantly higher in the PH group. Compared to the CHT Group, the PH group presented significantly lower values for the PP (p-value = 0.03), cSBP (p-value = 0.000), AI75% (p-value = 0.002) and PWV (p-value = 0.000).

Regarding nocturnal dipping of the SBP, a significant difference was observed between the PH and CHT groups (p-value = 0.003), and between the NT and CHT groups (p-value = 0.005), but not between the NT and PH groups. As for the diastolic nocturnal dipping, there was a significant difference only between the PH and CHT groups (p-value = 0.0006).

Discussion

The present study showed biochemical differences between the groups in respect to glycemia, creatinine, uric acid, eGFR, total cholesterol, HDL-c, LDL-c, and

triglycerides. By ABPM, there were differences between the groups in the three different periods (24-h, wakefulness and sleep) for the peripheral (SBP, DBP, MAP, PVR) and central parameters (cSBP, cDBP, AI75% and PWV).

Of the results obtained, the difference in gender of the NT and CHT groups compared to the PH group was previously observed and attributed to biological and behavioral factors [33]. Similar studies indicate that men generally have higher BP levels than premenopausal women, but after menopause, increases in BP levels in women makes this difference less or even nonexistent [34-36]. Thus, as hormone replacement therapy does not significantly reduce BP in most postmenopausal women, studies suggest that estrogen loss is not the only factor involved in elevating BP in this population [35,36].

On comparing biochemical parameters, significant differences were identified in the uric acid, creatinine and HDL-c levels in prehypertensive compared to normotensive subjects and significant differences were observed in glycemic, total cholesterol, LDL-c, and eGFR levels in relation to hypertensive patients. Triglyceride levels differed between the PH Group and both the NT and CHT groups. In relation to uric acid, the end product of the purine metabolism, evidence suggests that higher levels of uric acid are associated with a higher incidence of prehypertension [37-41]. However, although it contributes to the development of hypertension, the relationship between increased levels of uric acid in prehypertensive patients remains obscure in view of differences between results [38,43]. In this context, Vucak et al. did not observe an association between hyperuricemia and prehypertension; the association was significant only in subjects with high BMIs and triglycerides, suggesting that the interaction between these metabolic factors affects this relationship [42]. Thus, the present study corroborates the findings of Wang et al [38] and Liu et al [37]. In the latter, the authors conducted a prospective cohort study and reported

a positive correlation between uric acid levels and the incidence of prehypertension during a 6-year follow-up of a seemingly healthy population. The mechanisms underlying the increased risk of prehypertension in individuals with hyperuricemia require further investigation [37,39,43].

Another important finding, although the statistical analysis showed no significant difference, was that the prehypertensive subjects presented, on average, higher levels of uric acid than the hypertensive patients. One possible explanation is that the hypertensive patients of the present study were being followed-up and had controlled BP levels. This is supported by studies in which uric acid-reducing drugs were administered to prehypertensive individuals and, as a result, there was also a reduction in BP levels [44-46].

The prehypertensive subjects in this study had higher levels of triglycerides, total cholesterol and LDL-c compared to the NT and CHT groups and lower levels of HDL-c. These results corroborate other studies regarding normotensive individuals [47-50]; however, in relation to the CHT group, the result obtained may be explained by the use of statins [51]. Several studies have demonstrated the efficacy of statins in reducing cholesterol, LDL-c levels, as well as reducing the risk of CVD by inhibiting and regressing coronary atherosclerosis, stabilizing atherosclerotic plaques and consequent anti-inflammatory properties, and reducing complications and cardiovascular mortality [52-54]. Moreover, studies have sought to identify the effects of statins in individuals with low risk of vascular events, the results of which suggest that their indication should be reviewed [55]; in this sense, there is a possibility of extending benefits to prehypertensive individuals as prevention.

In the present study, renal function was evaluated using the MDRD and Cockcroft-Gault equations [56]. There were statistical differences for both equations on comparing the PH and CHT groups. This result was expected and it is in agreement with other studies due to the close relationship between hypertension and renal disease [57,58]. Another aspect to be stressed was that the results for the eGFR were lower, albeit not statistically significant, in the PH group than in the NT group. In this context, the present study does not corroborate the findings of Eriksen et al [59], who found that elevated BP is not associated with a decline in the eGFR in the general middle-aged population; however, the participants in the study differed in gender and age and, the authors grouped hypertensive and non-hypertensive individuals together. Thus, despite the small number of participants in the current study, these results suggest that prehypertensive patients already show a tendency of reduced renal function when compared with normotensive subjects.

In the present study, the comparison of 24-h peripheral and central variables by ABPM in prehypertensive patients showed significant differences in relation to the NT group regarding SBP, DBP, MAP, cSBP, cDBP and AI75%. These results highlight the importance of the follow-up and, in some cases, the early establishment of pharmacological treatment for these patients in order to prevent the development of AH [60,61]. The therapeutic approach would still be justified because of the difficulty to control pressure levels after the diagnosis of AH, as well as the greater risk of target organ lesions [62]. In this sense, the differences observed in cSBP, cDBP and AI75% between the PH and NT groups corroborate the findings of Hui et al [63], who demonstrated an association between higher central pressure and carotid intima-media thickness in prehypertensive patients and Hong et al [64], who showed a higher risk of atherosclerotic

plaques in these patients. In addition, Mousa et al [65] positively correlated central BP with increased left ventricular mass in normotensive and prehypertensive patients. Thus, the present study contributes to the characterization of prehypertension by ABPM, suggesting a need for more research with a larger sample size in order to diagnose and stratify cardiovascular risk in these patients. Moreover, this study recommends early follow-up, so much so that it showed that the cDBP in the PH group differed from the NT group during wakefulness and sleep.

Associated with this, we observed a difference in the PP, AI75% and PWV between prehypertension and hypertension in the three different periods (24-h, wakefulness and sleep). These data corroborate previous studies that suggest that prehypertension is associated with an acceleration of arterial stiffness, which, if it persists, will contribute to the development of AH [66,67]. As the PWV is a predictor for cardiovascular events and mortality in hypertensive patients, its use may be beneficial to prevent the development of sustained hypertension [67].

Limitations

The study population consisted of a relatively small sample of prehypertensive volunteers (n = 39), which may limit the generalization of the results. In addition, our cross-sectional findings need to be confirmed in longitudinal studies, including the identification of the predictive variables obtained by ABPM. Another important factor to consider is the method of evaluation of the central hemodynamic parameters, as in this study a 24-hour monitoring device was used, while applanation tonometry was used in many published studies [66,67].

Conclusions

In summary, differences in ABPM were observed between the groups in the three periods (24-h, wakefulness and sleep) for peripheral (SBP, DBP, MAP) and central (cSBP, cDBP, PVR, AI75% and PWV) hemodynamic parameters. The PH group presented higher values of peripheral and central pressure compared to the NT group and lower values compared to the CHT group in all periods. These results suggest that in prehypertension there are already functional and structural alterations that predispose subjects to the development of hypertension.

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Conflict(s) of Interest/Disclosure(s)

The authors declare that there is no conflict of interest.

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Table 1: Clinical and biochemical parameters in normotensive, prehypertensive and controlled hypertensive individuals.

Variable	NT (n = 47)[a]	PH (n = 39)[b]	CHT (n = 138)[c]	*p*-value (axbxc)	a x b	a x c	b x c
Age (years)	48.3 ± 10.6	50.1 ± 9.6	57.7 ± 10.9	0.000#	NS	0.000#	0.000#
Male (%)	9 (19.1)	34 (85)	61 (43.5)	0.000#	0.000#	0.001#	0.000#
White (%)	42 (89.3)	38 (97.4)	121 (87.6)	NS	-	-	-
BMI (kg/m^2)	27 ± 6.1	27.7 ± 4.4	29.4 ± 5.0	0.007#	NS	0.01*	NS
Smokers - n (%)	3 (6.3)	5 (12.8)	12 (8.6)	NS	-	-	-
Diabetes - n (%)	1 (2.1)	0	41 (29.7)	0.000#	NS	0.000#	0.000#
Biochemical parameters							
Glycemia (mg/dL)	97 ± 1.2	92.8 ± 9.7	119.6 ± 1.4	0.000#	NS	0.000#	0.000#
Creatinine (mg/dL)	0.75 ± 0.1	0.90 ± 0.1	0.96 ± 0.4	0.000#	0.000#	0.000#	NS
UA (mg/dL)	3.9 ± 0.9	6.1 ± 1.2	5.7 ± 2.0	0.000#	0.000#	0.000#	NS
eGFR (MDRD)	95.8 ± 20.8	91 ± 20.9	76.8 ± 20.6	0.000#	NS	0.000#	0.01*
Potassium (mEq/L)	4.3 ± 0.4	4.4 ± 0.2	4.4 ± 0.2	NS	-	-	-
TC (mg/dL)	195.4 ± 32.5	204.4 ± 26	184.6 ± 45.4	0.001*	NS	NS	0.000#
HDL-c (mg/dL)	57.7 ± 13	45 ± 6.4	47.9 ± 14.6	0.000##	0.000#	0.000#	NS
LDL-c (mg/dL)	114.9 ± 33.5	124.1 ± 27.6	106.1 ± 41.2	0.003*	NS	NS	0.002*
TG (mg/dL)	110.9 ± 40.5	169 ± 63	149.7 ± 102.5	0.000#	0.000#	0.000#	0.02*
Drugs - n (%)							
Statins	-	-	44 (31.8)				
Diuretics	-		39 (28.2)				
ARB	-		67 (48.5)				
ACEi	-		42 (30.4)				
CCB	-		46 (33.3)				
B-blockers	-		34 (24.6)				
Antiaggregant and/or anticoagulant	1 (2.12)	-	19 (13.7)	NS			

NT: normotensive; PH: prehypertensive; CHT: Controlled hypertensive; BMI: body mass index; eGFR: estimated Glomerular filtration rate; MDRD: Modification of Diet in Renal Disease; UA: Uric acid; TC: Total cholesterol; HDL-c: High-density lipoprotein; LDL-c: Low-density lipoprotein; TG: Triglycerides; ARB: Angiotensin receptor blockers; ACEi: Angiotensin converting enzyme inhibitors; CCB: Calcium channel blockers; B-blockers: Beta-blockers

**p <0.05; #p <0.001*; NS - non significant

Table 2: Peripheral and central hemodynamic parameters in normotensive, prehypertensive and controlled hypertensive individuals.

Period	Variable	NT (n = 47)[a]	PH (n = 39)[b]	CHT (n = 138)[c]	p-value	a x b	a x c	b x c
24-h	SBP	109 ± 6.2	117.4 ± 7.3	123.7 ± 12.5	0.000#	0.000#	0.000#	0.02*
	DBP	67 ± 6.8	74.3 ± 7.5	76.5 ± 10.8	0.000#	0.000#	0.000#	NS
	MAP	85.8 ± 6.4	94.1 ± 6.5	98 ± 10.9	0.000#	0.000#	0.000#	NS
	HR	75.8 ± 8	73.5 ± 8.9	74.3 ± 10.4	NS	-	-	-
	PP	42.0 ± 5.7	42.7 ± 6.2	47.2 ± 9.0	0.000#	NS	0.001#	0.02*
	cSBP	100.4 ± 12	109.2 ± 6.8	114.9 ± 11.3	0.000#	0.000#	0.000#	0.01*
	cDBP	66.9± 11.8	75 ± 7.2	78 ± 11.1	0.000#	0.002*	0.000#	NS
	AI75%	24.9 ± 5.9	19.5 ± 6.9	24.9 ± 9.2	0.001#	0.008*	NS	0.001*
	CO	3.9 ± 0.3	4.0 ± 0.4	4.1 ± 0.5	0.02*	NS	0.01*	NS
	PVR	1.24 ±0.09	1.28 ± 0.11	1.33 ± 0.15	0.001*	NS	0.000#	NS
	PWV	6.7 ± 1.24	7.0 ± 1.11	8.2 ± 1.46	0.000#	NS	0.000#	0.000#
Wakefulness	SBP	125.0± 6.5	121.3 ± 8.1	125.9 ± 12.6	0.000#	0.000#	0.000#	NS
	DBP	70.4 ± 6.9	78.8 ± 7.8	79.0 ± 11.3	0.000#	0.000#	0.000#	NS
	MAP	89.6 ± 5.9	98.3 ± 7.1	100.6 ± 11.0	0.000#	0.000#	0.000#	NS
	HR	79.7 ± 9.0	78.2 ± 9.1	77.9 ± 11.1	NS	-	-	-
	PP	42.0 ± 6.3	42.7 ± 6.6	46.8 ± 9.0	0.001*	NS	0.003*	0.03*
	cSBP	104.1± 5.7	112.3 ± 8.0	116 ± 11.5	0.000#	0.000#	0.000#	NS
	cDBP	72.3 ± 7.5	80.3 ± 7.9	81.0 ± 11.5	0.000#	0.000#	0.000#	NS
	AI75%	25.9 ± 6.8	20.8 ± 6.5	25.3 ± 8.7	0.002*	0.006*	NS	0.004*
	CO	3.9 ± 0.2	4.2 ± 0.3	4.1 ± 0.4	0.001*	0.006*	0.002*	NS
	PVR	1.27± 0.08	1.30 ± 0.11	1.34 ± 0.13	0.005*	NS	0.004#	NS
	PWV	6.8 ± 1.24	7.1 ± 1.12	8.3 ± 1.37	0.000#	NS	0.000#	0.000#
Sleep	SBP	103.1± 8.0	110.7 ± 8.1	119.9 ± 13.7	0.000#	0.01*	0.000#	0.000#
	DBP	61.3 ± 8.1	67.5 ± 8.2	72 ± 11.3	0.000#	0.01*	0.000#	NS
	MAP	80.6 ± 7.4	87.3 ± 7.3	93.9 ± 11.5	0.000#	0.007*	0.000#	0.006*

HR	69.1 ± 7.7	66.2 ± 9.6	68.3 ± 9.9	NS	-	-	-
PP	41.8 ± 5.8	43.0 ± 6.5	47.7 ± 9.4	0.000#	NS	0.001*	0.03*
cSBP	98.6 ± 8.3	104.2 ± 7.8	113.0 ± 12.7	0.000#	NS	0.000#	0.000#
cDBP	62.1 ± 8.0	68.3 ± 8.0	73.1 ± 11.6	0.000#	0.01*	0.000#	NS
AI75%	23.4 ± 8.5	17.4 ± 9.4	24.2 ± 11.5	0.003*	NS	NS	0.002*
CO	3.7 ± 0.4	3.8 ± 0.5	3.9 ± 0.5	NS	-	-	-
PVR	1.21± 0.14	1.25 ± 0.15	1.33 ± 0.18	0.000#	NS	0.001*	NS
PWV	6.5 ± 1.26	6.8 ± 1.11	8.17 ± 1.48	0.000#	NS	0.000#	0.000#

NT: normotensive; PH: prehypertensive; CHT: Controlled hypertensive; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean arterial pressure; HR: Heart rate; PP: Pulse pressure; cSBP: Central systolic blood pressure; cDBP: Central diastolic blood pressure; AI75%: Augmentation index; CO: Cardiac output; PVR: total vascular resistance; PWV: Pulse wave velocity

**p <0.05; #p <0.001*; NS - non significant

Table 3: Nocturnal dipping in normotensive, prehypertensive and controlled hypertensive individuals.

	NT (n = 47)[a]	**PH** (n = 39)[b]	**CHT** (n = 138)[c]	a x b	a x c	b x c
Non-Dipping Systolic (%)	29 (61.7%)	25 (64.1%)	113(81.8%)	NS	0.005[#]	0.003[#]
Non-Dipping Diastolic (%)	23 (48.9%)	12 (30.7%)	84 (60.8%)	NS	0.05	0.0006[#]

NT: normotensive; PH: prehypertensive; CHT: Controlled hypertensive

**p <0.05; #p <0.001*; NS - non significant

INFLUENCE OF ANTIHYPERTENSIVE TREATMENT ON MMP-9 LEVELS IN CONTROLLED HYPERTENSIVE PATIENTS

SUMMARY TABLE

WHAT IS KNOWN

- Ambulatory blood pressure monitoring can provide data on peripheral and central hemodynamic parameters associated with arterial stiffness.
- Imbalances between the mechanisms of activation and inactivation of metalloproteinase-9 (MMP-9) have been associated with vasomotor alterations. This enzyme participates in the degradation of components of the extracellular matrix. Thus, MMP-9 is involved to the development of cardiovascular disease and to the arterial stiffness process.

WHAT THIS STUDY ADDS

- Prehypertensive individuals present higher values of the peripheral and central hemodynamic parameters than the normotensive group, except heart rate and augmentation index.
- This study demonstrates that MMP-9 levels are higher in prehypertensive than in controlled hypertensive individuals, possibly due to anti-hypertensive treatment.
- There is correlation between MMP-9 levels and the heart rate and cardiac output parameters, observed by brachial oscillometry using the Mobil-O-Graph® system.

Abstract

Ambulatory blood pressure monitoring (ABPM), in addition to peripheral blood pressure (BP) measurements, provides data on central hemodynamic, such as pulse wave velocity (PWV), augmentation index (AI75%) and central pressure, which are associated with arterial stiffness. The development of arterial stiffness is related to an extracellular matrix enzyme called metalloproteinase-9 (MMP-9). The aim of the current study is to evaluate the correlation between the variables obtained by ABPM and levels of MMP-9 at different BP levels. On-hundred and one individuals were enrolled: 21 normotensive (NT), 36 prehypertensive (PH), and 44 controlled hypertensive (CHT). ABPM and MMP-9 levels were determined in all participants. Age of the participants ranged from 30-71 years. MMP-9 concentration was significantly higher in the PH (4.74 ± 0.5 ng/mL) compared to CHT group (4.41 ± 0.5 ng/mL) (p=0.02).The CHT group differed from the PH group in relation to MMP-9 levels (4.41 ± 0.5 ng/mL vs. 4.74 ± 0.5 ng/mL; p-value = 0.02). MMP-9 levels correlated with cardiac output and peripheral vascular resistance in the three periods (24 h, wakefulness and sleep) evaluated by ABPM. PWV measurements were greater in CHT than PH (8.1 ± 1.2 vs. 6.9 ± 1; p-value = 0.0003, respectively), but no differences were found in AI75% between CHT and PH groups (22.6 ± 8.9 vs. 19.8 ± 7.4; p-value = NS, respectively). Pre-hypertensive individuals had higher levels of MMP-9 than controlled hypertensive subjects, suggesting that antihypertensive therapy may reduce MMP-9 plasma levels.

Key words: Arterial hypertension; Prehypertension; Ambulatory blood pressure monitoring; Arterial stiffness; Metalloproteinase

Introduction

Ambulatory blood pressure monitoring (ABPM) is an evolution in the methods used to measure blood pressure (BP) as it provides a large number of measurements over 24 hours and is able to identify several clinical situations related to arterial hypertension [1,2].

In this context, the main uses of ABPM include the diagnosis of hypertension and borderline hypertension, white coat hypertension, resistant hypertension, nocturnal hypertension, gestational hypertension and masked hypertension. In addition, it evaluates therapeutic efficacy, stratifies cardiovascular risk and serves as a predictor for cardiovascular outcomes [1,3,4].

Some indicators allow to evaluate the relationship between the parameters measured during 24-h ABPM and target organ damage (TOD) [4]. Authors have indicated a greater association of systolic blood pressure (SBP) obtained by ABPM with TOD, such as left ventricular hypertrophy and stroke, than office SBP [5]. The use of ABPM is also effective in the detection of high-risk cardiovascular disease (CVD) [6]. Finally, ABPM identifies BP variations during sleep, especially in individuals whose BP does not have adequate nocturnal fall, thus being classified as 'non-dippers' [4,6,7].

On the other hand, ABPM may also approach arterial stiffness, which is independent predictor of CVD [8,9]. The parameters of arterial stiffness obtained by ABPM include pulse wave velocity (PWV), the augmentation index (AI) and central systolic BP (cSBP), and diastolic BP (cDBP) [10]. Arterial rigidity results from the early return of reflected waves from peripheral vessels to the ascending aorta artery during the systole rather than the diastole of the subsequent cardiac cycle.

The elastic properties of the wall vary throughout the arterial tree with this heterogeneity being associated with the molecular, cellular and histological structure of the arterial wall [10]. Imbalances between the mechanisms of activation and inactivation of metalloproteinase-9 (MMP-9) have been associated with vasomotor alterations. This enzyme participates in the degradation of components of the extracellular matrix, which in turn is implicated in the development of CVD and in the arterial stiffening process [11,12]. Thus, we aimed to correlate the peripheral and central hemodynamic variables obtained by ABPM and MMP-9 plasmatic levels.

Methods

Patients

This study was approved by the Research Ethics Committee of the Medical School. Subsequently, individuals were invited, informed and consulted about their interest and consented to participate as volunteers; regardless of their decision to participate or not, patients were informed that their ongoing treatment would not be prejudiced. The present study was carried out according to the norms of ethics of the Declaration of Helsinki.

One hundred and one subjects divided in three groups participated in this cross-sectional. The control group consisted of 21 untreated normotensive patients (NT) characterized by SBP <120 mmHg and DBP <80 mmHg. A prehypertensive group (PH, n=36) was defined by SBP values between 120 and 139 mmHg and/or DBP between 80 and 89 mmHg. The third group included 44 controlled hypertensive individuals (CHT) followed up at a university outpatient clinic specialized in hypertension with SBP <140 mmHg and DBP <90 mmHg when using antihypertensive drugs.

The exclusion criteria were pregnancy, low life expectancy, chronic diseases that could limit participation in the study (e.g. tumors), difficulty in comprehension, inability to measure BP and refusal to sign the written consent form. For NT participants the previous diagnosis of hypertension or previous use of antihypertensive drugs were also considered exclusion criteria.

Clinical and biochemical analysis

A questionnaire was used to collect information about the participant's history, associated diseases (diabetes mellitus), medications in use, smoking, and family history. Weight and height were measured using anthropometric scales. Peripheral blood was collected to investigate the biochemical profile (blood glucose, serum creatinine, uric acid, potassium and lipid profile). The lipid profile was assessed by measuring cholesterol (total and fractions) and triglycerides after 12-hours of fasting.

Metalloproteinase-9 quantification

Venous blood samples were also collected by venipuncture in vacutainer tubes with EDTA (Becton-Dickinson, São Paulo, Brazil) and centrifuged at 3500 revolutions per minute for ten minutes. The plasma fractions were immediately stored at -70°C until MMP-9 quantification. MMP-9 levels were measured using the human MMP-9 ELISA kit (R & D Systems, Inc., Minneapolis, MN, USA). Subsequently, MMP-9 values were transformed into negative logarithms to reflect normal distribution for statistical analysis.

Ambulatory Blood Pressure Monitoring (ABPM)

All participants were submitted to ABPM performed on a day of standard activity using a cuff adequate to the individual's arm size and the Mobil-O-Graph® 24h PWA Monitor (IEM, Stolberg, Germany) [13]. The parameters obtained by the device included SBP, DBP, mean artery pressure (MAP), heart rate (HR), pulse pressure (PP), cSBP, cDBP, AI corrected for heart rate of 75 bpm (AI75%), cardiac output (CO), peripheral vascular resistance (PVR), and pulse wave velocity (PWV). All variables were measured at 30-minute intervals, and means were obtained for three periods: 24 hours (24-h), wakefulness and sleep.

The nocturnal dip was standardized as a drop in SBP and DBP of between 10% and 20% from the period of wakefulness to the sleep period. Absence of dipping was considered when BP did not show a drop or there was an increase, or even when the decrease was attenuated (<10%), according to a report of the British Hypertension Society and the Brazilian Guidelines [14,15].

Statistical analysis

Data are presented as means ± standard deviation (SD) for continuous variables and percentages for categorical variables. Groups were compared using the ANOVA test for normal quantitative variables followed by the Tukey's test, and the Kruskal-Wallis test for nonparametric quantitative variables. The Pearson correlation test was also performed to investigate relationships between the variables obtained by ABPM in respect to MMP-9 levels. The chi-square or Fisher's exact test were used for qualitative variables. In all cases, significance was assumed for an alpha error of 5% (p-value <0.05).

All statistical analyses were performed using the SPSS computer program version 24.0 (SPSS Inc., Chicago, Ill., USA).

Results

Table 1 shows the demographic characteristics, including biochemical variables. Hypertensive individuals had a higher mean age compared to the NT and PH Groups. The CHT group also had a higher frequency of diabetes (25%) compared to the NT (0%; p-value = 0.008) and PH groups (5.5%; p-value = 0.01).

Regarding the biochemical parameters, serum levels of glucose, HDL-c and uric acid presented statistically significant differences between the CHT and NT Groups. The PH group also differed significantly from the NT group in relation to HDL-c, triglycerides and uric acid. PH had higher MMP-9 concentration compared with CHT (4.41 ng/mL vs. 4.74 ng/mL; p-value <0.05, respectively - Figure 1).

The results obtained by ABPM are shown in Table 2. In relation to the 24-hour period, the SBP, cSBP and PWV were significantly higher in the CHT group compared to the PH group, while in the wakefulness period only PWV was statistically higher in the CHT group. During the sleep period peripheral (SBP, PP) and central hemodynamic parameters (cSBP, PWV) were significantly higher in the CHT group compared to the PH group.

For statistical analysis, the participants of the three groups were divided into dippers and non-dippers and the variables PWV, AI75% and MMP-9 were compared between the groups. The PWV was higher in the non-dipper group, with significant differences in the 24 h and sleep periods (Table 3).

Table 4 shows the correlation analysis of ABPM parameters with MMP-9, which was performed by period for the three groups evaluated together, but taking the sample size of each group into consideration.

Discussion

The present study found differences in the peripheral and central hemodynamic variables obtained by ABPM between the PH group and the NT (SBP, DBP, MAP) and CHT groups (SBP, cSBP, PWV). The PH group presented higher values than the NT group for all ABPM variables except for HR and AI75% and lower values than the CHT group. Regarding MMP-9, there was a discrete positive correlation only with the HR and CO in the three periods assessed by the ABPM. The findings also showed greater biochemical levels (glycemia, LDL-c, triglycerides, total cholesterol) in PH individuals when compared to CHT, albeit without statistical significance.

In the present study, the differences in BP between the groups identified by ABPM corroborate studies that aimed to compare the BP values obtained in the office with those obtained by 24-hour monitoring. These studies found a better association between ABPM results and the development of CVD [16]. Currently, new non-invasive methods of evaluation of the peripheral BP together with central hemodynamic parameters offer a differentiated view of the arterial tree and, consequently, of TOD. In this context, the findings of the central hemodynamic parameters evaluated by ABPM (cSBP, cDBP and PWV) were progressively higher from NT to PH to CHT. This demonstrates the importance of measuring the central hemodynamic parameters in the clinical practice and shows that concern about pre-hypertension is justified, since PH individuals present with changes not only of the peripheral but also of central hemodynamic parameters.

Although the groups were not matched for age, the higher mean age found in the CHT group can be explained by the aging process itself; as age increases the prevalence of hypertension also increases [17]. Hypertension is a condition that is not only related to elevated BP as there is also an inflammatory substrate present in the arterial wall with the participation of a proteolytic process determined by metalloproteinases [18,19]. Thus, in relation to the levels of MMP-9, whose expression has been associated with the hypertensive process, arterial stiffness and the presence of CVD [11,12,20], no correlation was found with the peripheral and central hemodynamic variables monitored by ABPM with the exception of HF and CO.

These findings may be justified by the mechanical overload and sodium overload present in hypertension, which increases the activation of the gene and both arterial and circulating MMP-2 and MMP-9 levels, which play a central role in the pathogenesis of hypertension [21]. Thus, increases in mechanical stress and blood flow in the arterial wall may result in marked increases in MMP-2 and MMP-9 activity [22]. Elevations of arterial metalloproteinases associated to BP probably result from the activation of pro-inflammatory transcriptional factors, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [23]. Several authors have shown that activated MMP-2 contributes to increased BP by altering arterial wall homeostasis, elevating vasoconstrictors (big endothelin-1) and reducing vasodilators (endothelial nitric oxide synthase) [24]. Metalloproteinases, including MMP-9, appear to reduce the density of β2 adrenergic receptors in the arterioles and, consequently, increase arteriolar tone, a fact that contributes to the elevation of the BP [19,25].

This study demonstrated that MMP-9 levels were higher in PH subjects than in CHT patients. Angiotensin II (Ang II), which plays a central role in the pathogenesis of

hypertension, also activates arterial metalloproteinases. This condition was observed by Wang et al., which demonstrated elimination of metalloproteinases activation using an Ang II receptor antagonist [26]. Therefore, our finding of lower levels of MMP-9 may be justified by the use of antihypertensive agents by the CHT patients, a fact previously observed in other studies using candesartan, lisinopril and lercadipine [27-30].

Although the diet and physical activity were not investigated in this study, the biochemical-metabolic findings corroborate evidence that the association of eating habits and physical exercises may influence metabolic parameters (glycemia and lipids), which in turn appear to be associated with the development of higher blood pressure levels [31]. Thus, the PH subjects presented different lipid levels (higher total cholesterol, LDL-c and triglyceride levels and lower levels of HDL-c) in relation to the CHT group, possibly resulting from the effects of the statins that the CHT group were taking as part of their treatment [32].

The most important factor limiting the power of this study is the low number of patients, though most of the related studies in the literature have small numbers of patients as well [33-35]. Another limitation in the study groups was that the expression of TIMP-1, an MMP-9 inhibitor, was not evaluated, which could contribute to a better understanding of the relationship between MMP-9/TIMP-1 in the PH and CHT groups. Nevertheless, the results were significant. Even though this was a cross-sectional study, the patient group was carefully selected and thus we believe that it is a valuable contribution to the literature on PH and CHT individuals.

In summary, this study demonstrated that serum MMP-9 is higher in PH than in CHT subjects, a fact that be explained by use of antihypertensive drugs during treatment. On the other hand, the correlation of MMP-9 with HR and CO, found by ABPM, suggests

the important participation of MMP-9 as an underlying mechanism in hypertension.

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Conflict of Interest

The authors declared no conflict of interest.

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Table 1: Clinical characteristics and biochemical variables of normotensive, prehypertensive and controlled hypertensive subjects

Characteristic	**NT** (***n*=21**)[a]	**PH** (***n*=36**)[b]	**CHT** (***n*=44**)[c]	***p*-value**			
				(axbxcxd)	a x b	a x c	b x c
Age (years)	47.76±10.3	49.69±9.5	56.61±9.1	0.0008	NS	< 0.01	< 0.01
Gender (male; %)	5; 23.8%	24; 66.6%	25; 56.8%	-	0.002	0.01	NS
BMI (kg/m^2)	25±5.4	27.8±4.5	29.5±4.3	0.001	NS	0.0004	NS
Smoker (%)	3 (14.2%)	7 (19.4%)	7 (15.9%)	-	NS	NS	NS
History of diabetes (%)	-	2 (5.5%)	11 (25%)	-	NS	0.008	0.01
Skin color (White; %)	16 (76.1%)	32 (88.8%)	38 (86.3%)	-	NS	NS	NS
Statins (n, %)	-	-	14 (31.8%)	-	NS	0.001	0.0001
Biochemical parameters							
Fasting glucose (mg/dL)	84.9 ± 14.7	92.5 ± 11.5	103.6 ± 35	0.04	NS	< 0.05	NS
HDL-cholesterol (mg/dL)	60.3±16.7	46.1±8.9	48.3±11.6	0.001	0.001	0.001	NS
LDL-cholesterol (mg/dL)	113.3±34.2	129.4±33.8	121.6±37.6	NS	-	-	-
Total cholesterol (mg/dL)	198.6±28.5	206.8±31.1	201.1±41.9	NS	-	-	-
Triglycerides (mg/dL)	98.7±33.1	163.8±85.2	146.7±90	0.02	0.007	NS	NS
Serum creatinine (mg/dL)	0.84 ± 0.2	0.92 ± 0.1	0.94 ± 0.3	NS	-	-	-
Uric acid (mg/dL)	3.9±1.1	6.3±1.8	6.3±2	<0.0001	0.0001	< 0.0001	NS
Potassium (mEq/L)	4.2±0.4	4.4±0.3	4.3±0.5	NS	-	-	-

NT: normotensive group; PH: prehypertensive group; CHT: controlled hypertensive group; BMI: body mass index. NS - non-significant. Values are means ± Standard Deviation

Table 2: Peripheral and central hemodynamic parameters of ABPM (24 h, wakefulness and sleep) in normotensive, prehypertensive and controlled hypertensive subjects

Period	NT (*n*=21)[a]	PH (*n*=36)[b]	CHT (*n*=44)[c]	*p*-value (axbxcxd)	a x b	a x c	b x c
24-h							
SBP	111.6±9.5	118±7.3	124.8±12.9	0.0002	0.02	< 0.0001	0.03
DBP	69.6±10.4	75.3±6.4	78.7±10.3	0.003	0.02	0.0008	NS
MAP	87.9±10.4	94.8±6.2	99.8±11	0.0002	0.01	< 0.0001	NS
HR	74.4±5.9	73.5±9.1	74.2±10.9	NS	-	-	-
PP	42.1±6.2	42.6±6	46±7.5	0.04	NS	0.02	NS
cSBP	104.9±9.5	109.6±7.1	115.9±11.6	0.0006	NS	0.0002	0.02
cDBP	70.6±10.8	76±7.3	80.1±10.6	0.003	NS	0.0008	NS
AI75%	22.3±7	19.8±7.4	22.6±8.9	NS	-	-	-
CO	4.0±0.3	4.08±0.4	4.2±0.5	NS	-	-	-
PVR	1.25±0.1	1.29±0.1	1.33±0.1	NS	-	-	-
PWV	6.6±1.1	6.9±1	8.1±1.2	0.0000	NS	< 0.0001	0.0003
Non-Dipper	14 (66.6%)	20 (55.5%)	32 (72.7%)		NS	NS	NS
WAKEFULNESS							
SBP	115.1±8.7	122±7.9	127.5±12.4	0.0001	0.01	< 0.0001	NS
DBP	73.2±10	79.5±6.9	81.8±9.9	0.005	0.01	0.001	NS
MAP	92±9.1	99±6.6	102.9±10.2	0.0002	0.004	< 0.0001	NS
HR	78.1±6.4	78.1±9.4	78.2±11.4	NS	-	-	-
PP	41.8±6.2	42.5±6.3	45.6±8.1	NS	-	-	-
cSBP	107.4±8.7	112.9±7.8	117.8±11.2	0.0006	0.02	0.0001	NS
cDBP	74.9±10.5	81±7.4	84±9.9	0.003	0.02	< 0.05	NS
AI75%	23.5±7.7	21±7	22.9±8.2	NS	-	-	-
CO	4.09±0.3	4.24±0.3	4.3±0.4	NS	-	-	-
PVR	1.28±0.1	1.31±0.1	1.33±0.1	NS	-	-	-
PWV	6.7±1.1	7.1±1	8.1±1.1	< 0.0001	NS	< 0.05	< 0.05
SLEEP							
SBP	106±10.8	111.1±8.3	120.3±14.9	0.0002	NS	< 0.01	< 0.01
DBP	63.5±11.6	68.4±7.2	73.2±12.3	0.003	NS	< 0.05	NS
MAP	83±10.5	87.9±7	94.8±13	0.005	NS	< 0.01	< 0.05
HR	68±7	65.9±9.8	67.3±10.5	NS	-	-	-
PP	42.4±7.1	42.7±6.5	47±7.4	0.01	NS	0.02	< 0.05

cSBP	101.7±11.3	104.5±8.2	113.3±13.9	0.001	NS	< 0.01	< 0.05
cDBP	64.7±11.9	69.1±7.2	74.1±12.6	0.006	NS	< 0.01	NS
AI75%	20.6±9	17.9±9.6	21.6±11.6	NS	-	-	-
CO	3.84±0.6	3.82±0.5	4.0±0.6	NS	-	-	-
PVR	1.22±0.1	1.27±0.1	1.31±0.2	NS	-	-	-
PWV	6.5±1.1	6.8±1	7.9±1.1	< 0.0001	NS	< 0.01	< 0.01

NT: normotensive group; PH: prehypertensive group; CHT: controlled hypertensive group; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean artery pressure; HR: Heart rate; PP: Pulse pressure; cSBP: Central systolic blood pressure; cDBP: Central diastolic blood pressure; AI75%: Augmentation index; CO: Cardiac output; PVR: Peripheral vascular resistance; PWV: Pulse wave velocity

NS - non-significant. Values are means ± Standard Deviation

Table 3: MMP-9 levels, pulse wave velocity and augmentation index in dippers and non-dippers

	Dipper (n = 35)	**Non-Dipper (n = 66)**	***p*-value**
Nl MMP-9	4.54 ± 0.65	4.51 ± 0.6	NS
PWV 24 h	7 ± 1.16	7.62 ± 1.28	0.02
PWV wakefulness	7.18 ± 1.18	7.66 ± 1.27	NS
PWV sleep	6.73 ± 1.14	7.55 ± 1.29	0.002
AI75% 24 h	22.4 ± 8.32	21.1 ± 7.97	NS
AI75% wakefulness	23 ± 7.97	22 ± 7.6	NS
AI75% sleep	21.4 ± 10.6	19.4 ± 10.4	NS

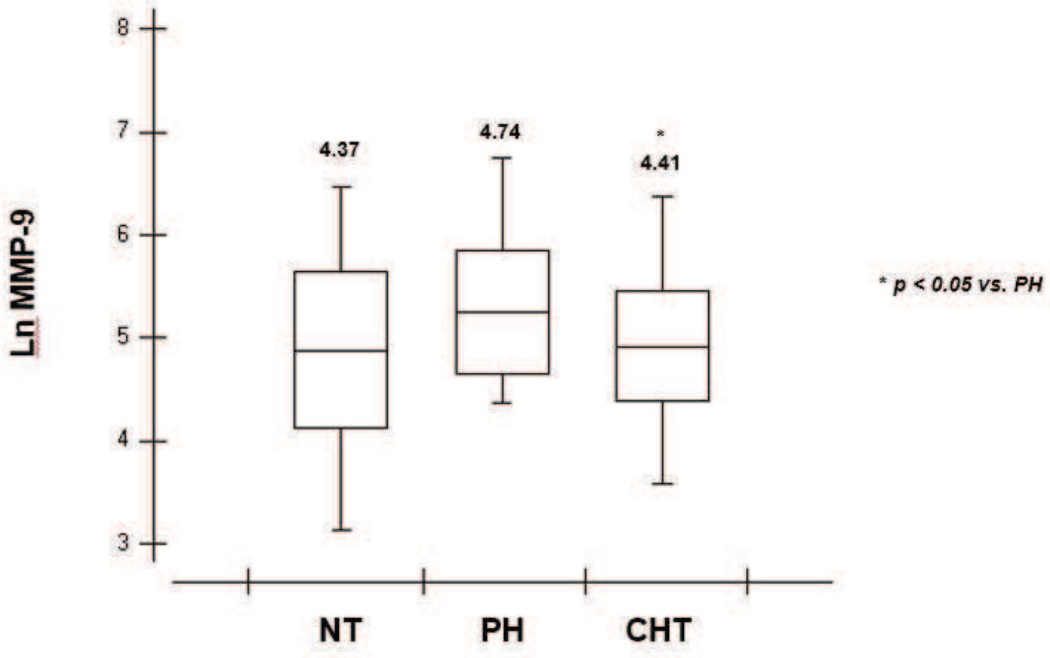
Nl: Negative Logarithm; MMP-9: metalloproteinase-9; PWV: Pulse wave velocity; AI75%: augmentation index

Table 4: Correlation of MMP-9 levels with ABPM variables (24 h, wakefulness and sleep) in the normotensive, prehypertensive and controlled hypertensive groups

Period	Variable	r	p-value
24h	SBP	0.099	NS
	DBP	0.088	NS
	MAP	0.096	NS
	HR	0.222	0.015
	PP	0.046	NS
	cSBP	0.102	NS
	cDBP	0.094	NS
	AI75%	-0.024	NS
	CO	0.199	0.030
	PVR	-0.100	NS
	PWV	-0.109	NS
WAKEFULNESS	SBP	0.087	NS
	DBP	0.063	NS
	MAP	0.082	NS
	HR	0.220	0.016
	PP	0.057	NS
	cSBP	0.080	NS
	cDBP	0.059	NS
	AI75%	-0.004	NS
	CO	0.247	0.007
	PVR	-0.142	NS
	PWV	-0.110	NS
SLEEP	SBP	0.106	NS
	DBP	0.110	NS
	MAP	0.113	NS
	HR	0.217	0.018
	PP	0.033	NS
	cSBP	0.112	NS
	cDBP	0.112	NS
	AI75%	-0.025	NS
	CO	0.204	0.026
	PVR	-0.069	NS
	PWV	-0.001	NS

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean artery pressure; HR: Heart rate; PP: Pulse pressure; cSBP: Central systolic blood pressure; cDBP: Central diastolic blood pressure; AI75%: Augmentation index; CO: Cardiac output; PVR: Peripheral vascular resistance; PWV: Pulse wave velocity; NS - non-significant

Figure 1: MMP-9 levels stratified by normotensive, prehypertensive and controlled hypertension groups



3 Conclusões

3. CONCLUSÕES

Os níveis de metaloproteinase-9 aumentam progressivamente à medida que os níveis de pressão arterial aumentam, ou seja, são progressivamente maiores nos grupos de normotensos, hipertensos controlados, de urgência e de emergência hipertensiva. Apesar do papel da MMP-9 na fisiopatologia da hipertensão não ser totalmente elucidado, sua avaliação pode representar um novo biomarcador em indivíduos com crise hipertensiva. Além disso, destaca-se que o ácido úrico também parece influenciar os níveis de metaloproteinase-9, o que sugere o aumento da atividade inflamatória presente na elevação aguda da pressão arterial.

Outro importante achado consiste no fato dos indivíduos pré-hipertensos apresentarem níveis mais elevados de metaloproteinase-9 em comparação a hipertensos controlados, sugerindo que o uso de anti-hipertensivos pode inibir a ativação dessas enzimas e, consequentemente, sua participação na degradação de componentes da matriz extracelular.

No estudo de correlação dos níveis de metaloproteinase-9 e os parâmetros obtidos pela MAPA houve apenas fraca correlação positiva com a frequência cardíaca e o débito cardíaco no período de 24 horas, vigília e sono. Entretanto, com a MAPA foi possível identificar valores progressivamente mais elevados e diferenças nas variáveis periféricas e centrais ao comparar indivíduos pré-hipertensos a normotensos (PAS, PAD, PASc, PADc, PAM e AI75%) e hipertensos controlados (PAS, PASc, PP, AI75% e VOP), evidenciando, portanto, que no estado de pré-hipertensão já existem alterações da hemodinâmica periférica e central. Além disso, esses achados contribuem para a caracterização da pré-hipertensão pela MAPA e reforçam a importância do acompanhamento precoce desse grupo, especialmente pelas diferenças entre pré-

hipertensos e normotensos em relação à pressão central (nos períodos de vigília e sono), e em relação a hipertensos controlados pelas diferenças dos marcadores de rigidez arterial (AI75% e VOP), sugerindo a associação da pré-hipertensão a uma aceleração do processo de alteração da estrutura vascular.

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