

Changes in metalloproteinases in healthy normotensive patients with high-normal blood pressure

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ABSTRACT. *Introduction.* High-normal blood pressure (HNBP) seems to be related to increased cardiovascular risk in healthy, normotensive subjects, while essential hypertension is associated with an increase in extracellular matrix content, especially fibrillar collagen type I. The aim of our study was to investigate whether collagen degradation is altered in healthy normotensives with HNBP, and whether this alteration could be related to disturbances in the matrix metalloproteinases plasma concentration, and to compare the findings to those of healthy normotensives with normal blood pressure (NBP) levels, matched for age, sex and BMI. *Methods.* Twenty six (14 males, 12 females) healthy, normotensive patients with HNBP, mean age 52 ± 5 yrs, and BMI 23 ± 1.5 kg/m² (group A), and 24, healthy normotensive patients (13males, 11females) with NBP, mean age 53 ± 6 yrs, and BMI 23.2 ± 1.4 kg/m² (group B), were studied. The two groups were matched for age, sex and BMI. Plasma levels of matrix metalloproteinase-9 (MMP-9) and tissue inhibitors (TIMP-1) and (TIMP-4) were determined by relevant ELISA in the study population. *Results.* Plasma MMP-9 levels were significantly higher, while TIMP-1 and TIMP-4 levels were significantly lower in group A compared to group B, (MMP-9 579 ± 147 versus 294 ± 111 ng/mL, TIMP-1 178 ± 45 versus 237 ± 35 ng/mL $p < 0.01$, and TIMP-4 2.2 ± 1.4 versus 4.4 ± 2.1 $p < 0.04$ respectively). *Conclusions.* Our findings suggest that healthy normotensives with high-normal blood pressure have significantly increased MMP-9 and decreased TIMP-1 and TIMP-4 plasma levels compared to healthy normotensives with normal blood pressure. These findings need further investigation.

Keywords: high normal blood pressure, metalloproteinases MMP-9, TIMP-1, TIMP-4

It is recognised that cardiovascular risk increases linearly at blood pressure levels lower than those that trigger the administration of antihypertensive therapy, and more specifically at a systolic pressure of 130-135 mmHg and a diastolic pressure of 80-85 mmHg. It is also well known from the literature that patients with high-normal blood pressure (defined as systolic pressure 130-139 mmHg and/or diastolic pressure 85-89 mmHg), have higher rates of cardiovascular events than those with normal blood pressure [1].

Matrix metalloproteinases (MMPs) are zinc-dependent endoproteinases with the combined ability to degrade all the components of extracellular matrix (ECM) at physiological pH [2]. They are involved in a variety of physiological and pathological processes related to connective tissue turnover, remodelling, angiogenesis and atherosclerosis [3], and their activity is tightly controlled by endogenous inhibitors such as the tissue inhibitors of metalloproteinases (TIMPs) [4].

The aim of this study was to test the hypothesis that disturbances plasma levels of matrix metalloproteinase-9 (MMP-9) and tissue inhibitors of matrix metalloproteinase-1 (TIMP-1) and 4 (TIMP-4), exists in healthy,

normotensive subjects with high-normal blood pressure (HNBP) compared to healthy normotensives with normal blood pressure (NBP) with no history of cardiovascular disease. To the best of our knowledge, this is the first study addressing plasma levels of MMP-9, TIMP-1, and TIMP-4 in HNBP patients.

METHODS

Two groups of subjects were studied: group A comprised twenty-six (14 males, 12 females), healthy normotensives with HNBP, mean age 52 ± 5 years old and body mass index (BMI) 23 ± 1.5 kg/m², and group B comprised 24, healthy normotensives, (13 males, 11 females) with NBP, mean age 53 ± 6 yrs and BMI 23.2 ± 1.4 kg/m² with no history of cardiovascular disease or diabetes mellitus. The demographic characteristics of the participants, as well as the variables included in the recent guidelines of the European Society of Hypertension to assess global cardiovascular risk, are presented in *table 1*. The classification of the participants was made by blood pressure measurements according to the 2003 European Society of Hypertension - European Society of Cardiology guidelines for the management of arterial hypertension criteria [5].

Table 1
Demographic characteristics and laboratory assessment of the study population

	Group A n = 26	Group B n = 24	p value
Age (years)	52 ± 5	53 ± 6	NS
BMI (kg/m ²)	23 ± 1.5	23.2 ± 1.4	NS
Sex (male/ female)	14/12	13/11	NS
Currently smoking	-	-	-
Tchol mg %	236 ± 25	235 ± 23	NS
HDL-C mg %	40 ± 6	41 ± 5	NS
Glucose mg % (fasting)	95 ± 7	93 ± 6	NS
Serum creatinine mg %	0.9 ± 0.2	0.88 ± 0.3	NS

The participants from both groups were taking no medication and were non-smokers. All subjects were following a standardized diet before sampling, and none of them had any thyroid function abnormality. Alcohol consumption was expressed in grams per day, and determined by a detailed questionnaire. Information concerning physical activity was obtained from a previously described questionnaire [6]. Before the study, written informed consent was obtained from each participant, which was approved by the hospital review committee. At the base-line examination, all participants underwent a physical examination with medical history, laboratory assessment of risk factors for cardiovascular disease, and routine electrocardiography.

Measurement of blood pressure and laboratory assessment

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at the time of the first and fifth Korotkoff sounds, respectively. Measurements were made on the right arm to the nearest millimetre of mercury using a mercury column sphygmomanometer. All measurements were made in the supine position after the patient had rested for 15 minutes. Results are the average of measurements obtained on at least three separate occasions, which were performed by the same trained nurse, who was not aware of the history of the subjects. At the base-line examination, the subjects were classified into one of the two, non-hypertensive blood pressure categories on the basis of the criteria of the ESH/ESC [5]. If the systolic and diastolic pressure readings fell into different categories, the higher of the two readings was used to assign the blood pressure category [7].

Blood sampling was performed after 12 hours of fasting, at 8 to 9 am, to determine plasma levels of MMP-9, TIMP-1, and TIMP-4. Venous blood was collected, in the absence of anticoagulant, into plastic tubes and was allowed to clot for 30 min at 25 °C. The clot was removed by centrifugation at 3000r/min for 15 min at 4°C, and the serum was aliquoted and stored at -20 °C until analysed (less than one month after collection).

Measurement of matrix metalloproteinases and their inhibitors

Assays for both matrix metalloproteinases and their inhibitors were performed concurrently to minimize any effects of repeated freeze-thaw cycles. Quantitative determinations of human MMP-9 and human TIMP-1 were per-

formed in duplicated using commercially available, enzyme-linked immunosorbent assay kits (R&D Systems Inc., Minneapolis, USA). Intra-assay and inter-assay coefficients of variation were, 2.3% and 7.5% for MMP-9, 4.4% and 4.2% for TIMP-1, respectively.

Quantitative determination of the MMP-9/TIMP-4 complex was performed in duplicated using the human MMP-9/TIMP-4 complex Duoset, (R&D Systems Inc., Minneapolis, USA), in accordance with the manufacturer's protocol.

The TIMPs (TIMP-1 to TIMP-4) can each form a high affinity complex with MMPs in a 1:1 stoichiometry [8, 9]. As the levels of MMP-9 were measured, as indicated above, the levels of tissue inhibitor of metalloproteinase-4 (TIMP-4) were calculated using the ratio MMP-9/TIMP-4. Investigators performing the assays were not aware of the identity of the samples studied. Results are reported as concentrations of MMP-9, TIMP-1 and TIMP-4 complex (ng/mL) present in samples.

Statistical analysis

Values are expressed as mean ± SD. An unpaired Student's *t*-test was used to assess differences between the two groups. A *p* < 0.05 was considered statistically significant. All analyses were performed with the SPSS statistical package.

RESULTS

As shown in *table 1*, there were no statistical differences according to age, sex, BMI or the other variables of cardiovascular risk, between the two groups. No differences were found concerning physical activity or alcohol consumption (data not shown). As shown in *table 2* and depicted in *figures 1, 2, 3*, plasma MMP-9 levels were significantly higher, while TIMP-1 and TIMP-4 plasma levels were significantly lower in group A compared to group B (MMP-9 579 ± 147 *versus* 294 ± 111 ng/mL, TIMP-1 178 ± 45 *versus* 237 ± 35 ng/mL *p* < 0.01, and TIMP-4 2.2 ± 1.4 *versus* 4.4 ± 2.1 ng/mL *p* < 0.04 respectively).

DISCUSSION

The current study demonstrated that in healthy, normotensive subjects with HNBP, plasma levels of MMP-9 were higher while TIMP-1 and TIMP-4 plasma levels were

Table 2
Results and comparison between groups

	Group A n = 26	Group B n = 24	p value
SBP (mmHg)	133 ± 2	126 ± 3	0.001
DBP (mmHg)	87 ± 2	82 ± 2	0.001
MMP-9 (ng/mL)	579 ± 147	294 ± 111	< 0.01
TIMP-1 (ng/mL)	178 ± 45	237 ± 35	< 0.01
TIMP-4 (ng/mL)	2.2 ± 1.4	4.4 ± 2.1	< 0.04

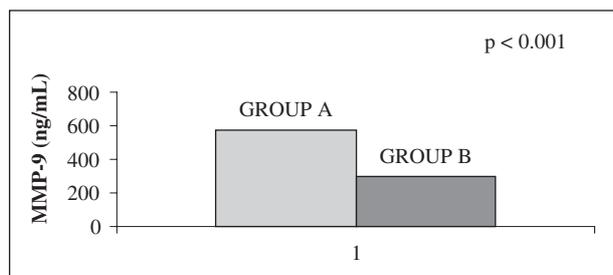


Figure 1

MMP-9 plasma levels in healthy normotensives with HNBP (group A) and healthy normotensives with NBP (group B).

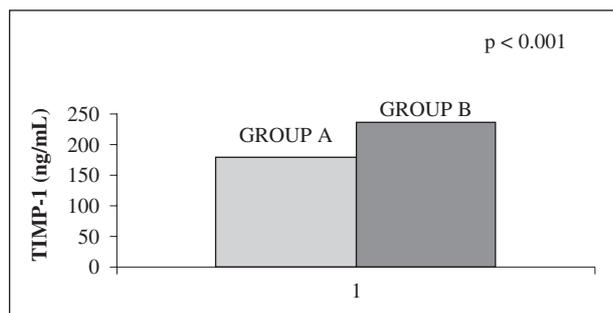


Figure 2

TIMP-1 plasma levels in healthy normotensives with HNBP (group A) and healthy normotensives with NBP (group B).

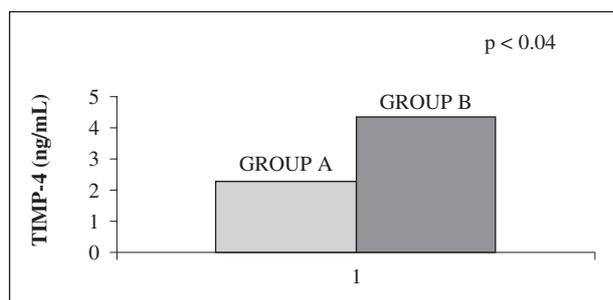


Figure 3

TIMP-4 plasma levels in healthy normotensives with HNBP (group A) and healthy normotensives with NBP (group B).

lower, compared to healthy normotensives with NBP, even when age, sex, BMI and the other variables of cardiovascular risks were matched for the two groups.

The extracellular matrix (ECM) is a vital component of the connective tissue surrounding the cells of solid organs. The ECM is composed of basic structural elements such as collagen and elastin, and more specialized proteins (e.g. fibrillin and fibronectin) and proteoglycans. Collagen

types I and III are the predominant constituents of the cardiovascular ECM. The breakdown of collagen and the other components are mediated by MMPs [3]. These enzymes are specifically regulated by TIMPs [10]. Hypertension in particular, is associated with an increase in ECM deposition especially of collagen types I, II, IV [3].

MMP-9 is a member of the group of gelatinases, while TIMP-1 is synthesized by most connective tissue cell types and has a broad spectrum inhibitory activity against most of MMPs. In actively resorbing tissues, TIMP-1 is highly expressed and forms high affinity, irreversible complexes with the active MMP enzymes [3]. TIMP-4 is the most abundant TIMP in human [11] and murine [12] hearts. Alteration in the balance between MMP and TIMP, resulting in enhanced MMP activity, has been shown to occur in long-term remodeling processes such as infarction [13], heart failure [14] and dilated cardiomyopathy [15].

Data from human studies have demonstrated a significant correlation between plasma levels of MMP-9 and TIMP-1, and blood pressure levels in many groups of normotensive and hypertensive patients. Laviades *et al.* noticed higher TIMP-1 plasma levels in patients with hypertension [16], and it has been suggested by Lindsay *et al.* that elevated TIMP-1 might be a useful marker of left ventricular diastolic function and fibrosis [17]. MMP-9 has been reported as decreased or unchanged in patients with hypertension compared to normotensive controls [18, 19], while Tayebjee *et al.* have demonstrated elevated MMP-9 and TIMP-1 plasma levels in hypertensive patients compared to normotensive controls. Additionally, the same authors noticed that elevated MMP-9 plasma levels seem to be related to higher Framingham cardiovascular risk scores [20] and that plasma levels of MMP-9 and TIMP-1 seem to be altered in gestational hypertension [21]. Furthermore, Tayebjee *et al.* have shown significantly higher MMP-9 and TIMP-1 levels in diabetic patients, compared to non-diabetics [22].

In accordance with these findings, our results showed significantly increased plasma levels of MMP-9. However, our results highlighted decreased plasma levels of TIMP-1, which is in contrast with the above results. This discrepancy in expression of MMP-9, and TIMP-1 may prove to be an early indicator of the future development of heart failure [23, 24].

TIMP-4 is a recently discovered, tissue inhibitor of metalloproteinase and is the most abundant in the human heart [11]. Recent experimental studies in hypertensive hearts have shown an increased expression of TIMP-4 in the medulla of the kidney that may inhibit the collagenolytic activity that contributes to glomerular injury and hypertensive remodeling [25]. Data from human studies in patients with cardiovascular disease have shown a selective down-

regulation of TIMP-4, along with upregulation of MMP-9 and gelatinolytic activity in the failing heart, alterations that favor matrix degradation and turnover [14]. This imbalance between plasma levels of MMP-9 and TIMP-4 is in agreement with our results and support the practice of new, target therapies that attempt to limit the processes of left ventricular remodeling and dilatation in the failing human heart [14].

A limitation of our study is that we did not perform tissue sampling in our study population, which ideally could have established a correlation between arterial or cardiac tissue activity and circulating MMP-9 and TIMP-1 levels. Animal studies in models with established hypertension, may be required in order to find out the links between structure- function, and local, as well as circulating MMP and TIMP levels.

In addition, we acknowledge that our sample size is relatively small, but it is broadly similar to the number of patients studied in previous works [16, 19]. Moreover, the net level of MMP activity partly depends on the relative concentrations of free active enzyme and TIMP [26], and both MMP and TIMP can circulate as free or complex forms, which are not readily distinguishable by ELISA.

CONCLUSIONS

Our findings suggest that healthy normotensives with HNBP have significantly higher MMP-9 plasma levels and significantly lower TIMP-1 and TIMP-4 plasma levels compared to healthy normotensives with NBP. This discrepancy in expression of MMP-9, TIMP-1 and TIMP-4 may trigger and promote LV remodeling and dysfunction in healthy normotensives with high-normal blood pressure. Further studies are necessary to confirm these findings, which support the practice of carefully examining healthy normotensives with HNBP in attempt to categorize them according to their cardiovascular risk, as a basis for the prevention of future heart disorders.

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