

## ORIGINAL ARTICLE

## Masked hypertension unfavourably affects haemostasis parameters

D. P. PAPADOPOULOS<sup>1</sup>, C. THOMOPOULOS<sup>2</sup>, I. MOUROUZIS<sup>1</sup>, A. KOTROTSOU<sup>1</sup>,  
E. SANIDAS<sup>1</sup>, U. PAPAACHOU<sup>2</sup>, M. DASKALAKI<sup>2</sup> & T.K. MAKRIS<sup>2</sup><sup>1</sup>ESH Excellent Center of Hypertension, Laiko University Hospital, Athens, Greece, <sup>2</sup>ESH Excellent Center of Hypertension, Elena Venizelou Maternity Hospital, Athens Greece**Abstract**

**Objective.** Recent evidence demonstrates that masked hypertension (MH) is a significant predictor of cardiovascular disease. The aim of our study was to examine the impact of MH on haemostasis parameters and to compare the findings to those of healthy normotensives matched for age, sex, body mass index and the rest of risk factors. **Design and method.** 130 (60 male, 70 female) healthy subjects mean age  $45 \pm 12$  years who had clinic blood pressure  $< 140/90$  mmHg were studied. The whole study population underwent 24-h ambulatory blood pressure monitoring (ABPM). According to the ABPM recordings, 24 individuals (eight males, 16 females) had MH (daytime systolic blood pressure  $\geq 135$  mmHg or daytime diastolic blood pressure  $\geq 85$  mmHg – group A) and the remaining 106 subjects (52 males, 54 females) had normal ABPM recordings – group B. Fibrinogen, thrombomodulin<sup>TM</sup>, the antigens of plasminogen activator inhibitor 1 (PAI-1Ag) and tissue plasminogen activator (tPA-Ag) were determined in the two groups. **Results.** The PAI-1 Ag, tPA-Ag, fibrinogen and TM levels were significantly higher in the masked hypertensive group than to normotensive control group. **Conclusions.** Our findings suggest that subjects with MH have significantly higher fibrinogen, TM, PAI-1Ag and tPA-Ag plasma levels compared with normotensives. This observation may have prognostic significance for future cardiovascular events in subjects with MH and needs further investigation.

**Key Words:** Fibrinogen, hypertension, masked hypertension, plasminogen activator inhibitor 1, thrombomodulin, tissue plasminogen activator

**Introduction**

The phenomenon of masked hypertension (MH) defined as a clinical condition when patient office blood pressure (BP) is less than 140/90 mmHg but his/her ambulatory or home BP readings are in the hypertensive range (1,2). Different BP thresholds have been proposed to define MH, making it difficult to compare results from various studies. Indeed the prevalence of MH in the general population could be as high as 10%, whereas data obtained in several cross-sectional studies have demonstrated large differences with prevalence rates from a low of 8% to a high of 49% (3–6). A body of evidence indicates that MH is a significant predictor of cardiovascular disease. Data obtained from several cross-sectional studies reported that MH is associated with increased left ventricular mass index (7–9) and carotid intima-media thickness (10). Furthermore, in

longitudinal studies, MH was a strong predictor of cardiovascular outcome (11), mortality (12) and target organ damage (13,14).

It has been previously shown that essential hypertension is associated with abnormalities in haemostatic/fibrinolytic balance and endothelial function, indicated by alterations in plasma levels of fibrinogen, plasminogen activator inhibitor (PAI), tissue plasminogen activator (tPA) and thrombomodulin<sup>TM</sup> (15–18). Recent evidence is accumulating that many of these markers are predictors of future vascular events, both ischaemic heart disease and stroke (19,20). Only few previous studies have examined a potential association between MH and these parameters (21). The aim of our study was to investigate whether MH affects plasma levels of haemostatic/fibrinolytic and endothelial function markers, including plasminogen activator inhibitor-1

(PAI-1), tissue plasminogen activator antigen (tPA-Ag), fibrinogen and TM.

## Methods

This is a consecutively recruited cohort. A total of 285 patients that attended the Hypertension Clinic of our hospital were screened. All patients included in the study had clinic BP < 140/90 mmHg and were taking no anti-hypertensive medication or other medication that interferes with parameters measured (e.g. aspirin, clopidogrel etc.) and were non-smokers. All subjects were under standardized diet before sampling and none of them had any thyroid functional abnormality; 145 out of 285 patients met these criteria. Five patients out of 145 that were initially enrolled were excluded from the study because of inadequate ABPM recordings. Ten more subjects from the normotensive group were excluded because of inadequate blood samples. Finally, this study was included in 130 (60 males, 70 females) subjects, mean age  $45 \pm 12$  years.

The whole study population underwent 24-h ambulatory BP monitoring (ABPM). According to the ABPM recordings, 24 individuals had MH {19%} (daytime systolic BP, SBP  $\geq 135$  mmHg or daytime diastolic BP, DBP  $\geq 85$  mmHg – group A) and the remaining 106 subjects had normal ABPM recordings – group B. 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 I (22).

Alcohol consumption was determined by a questionnaire, which asked for the daily consumption of wine, liquor and beer; alcohol intake was expressed in grams per day. Information concerning physical activity was obtained from questionnaires that have been previously described (23,24). Before the study, written informed consent was obtained from each participant, which was approved

by the hospital review committee. At the baseline examination, all participants underwent a physical examination with a medical history, laboratory assessment of risk factors for cardiovascular disease and routine electrocardiogram. Subjects were weighed (kg), and height (m) was measured wearing only light clothing without their shoes. The body mass index (BMI) was calculated as weight/height<sup>2</sup> (kg/m<sup>2</sup>).

### Measurement of BP and laboratory assessment

SBP and 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 (mmHg) with the use of a mercury sphygmomanometer. All measurements were made in the supine position after the patient had rested for 15 min. 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.

The recruitment of MH subjects was made according to a document of the European Society of Hypertension Working Group on Blood Pressure monitoring that define individuals with MH those who have clinic BP < 140/90 mmHg and daytime SBP > 135 mmHg or daytime DBP > 85 mmHg. This document was confirmed from the 2007 published edition of the European Society of Hypertension guidelines (25). BP measurements consisted of clinic BP (see above), home BP (average of morning and evening measurements, semiautomatic device), and ABPM with Spacelabs 90207, which recorded BP every 20 min during daytime (between 10:00 and 20:00 h) and 40 min during night-time (between midnight and 06:00 h) for 24 h (3). Subjects recorded a daily action profile from which information about the precise times of sleeping and waking were obtained. The onset of sleep was identified as the time that the subject went to bed. The subjects were instructed to carry out normal daily activities during the monitoring period.

Venous blood samples were collected without stasis after a 10-min supine rest. Participants were instructed to avoid strenuous physical activity and not to smoke tobacco during the hour preceding this examination, which took place between 08:00 and 09:00 h. All subjects had fasted for at least 12 h. Blood sampling was performed to determine plasma levels PAI-1-Ag, tPA-Ag and TM with an enzyme linked immunosorbent assay (ELISA; Diagnostica Stago, Asnieres, France). Fibrinogen levels were measured with Claus technique. Serum cholesterol and triglyceride levels were determined by an enzymatic method and low-density lipoprotein (LDL) was calculated according to the Friedwald formula, since no subject had a triglyceride level higher than 400 mg/dl.

Table I. Demographic characteristics and standard laboratory tests of the study population.

	Group A (n = 24)	Group B (n = 106)	p
Age	46 $\pm$ 7	44 $\pm$ 6	0.2
Gender (M/F)	11/13	49/57	0.7
BMI (kg/m <sup>2</sup> )	25.9 $\pm$ 2.1	25.5 $\pm$ 2.4	0.42
SBP clinic (mmHg)	125 $\pm$ 8	124 $\pm$ 7	0.6
DBP clinic (mmHg)	80 $\pm$ 3	79 $\pm$ 4	0.18
Total cholesterol (mg/dl)	234 $\pm$ 26	232 $\pm$ 25	0.75
HDL (mg/dl)	43 $\pm$ 6	42 $\pm$ 4	0.43
LDL (mg/dl)	160 $\pm$ 30	156 $\pm$ 27	0.55
Triglycerides (mg/dl)	99 $\pm$ 31	102 $\pm$ 32	0.7

Group A includes patients with masked hypertension and group B normotensive controls. SBP, systolic blood pressure; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; DBP, diastolic blood pressure.

### Statistical analysis

Values are expressed as the mean  $\pm$  SD. Differences between groups were analysed with *t*-test or Mann-Whitney as appropriate. A *p*-value of  $< 0.05$  was accepted as statistically significant.

### Results

Clinic and ambulatory BP values are presented in Table I. Haemostatic/fibrinolytic parameters were determined in 26 patients with confirmed MH and 104 healthy normotensives. The two groups were not different with respect to age, gender, BMI, smoking status and lipid profile (Table I). No differences were observed between groups regarding physical activity, alcohol consumption and menopausal status (data not shown).

The haemostasis balance parameters for each group are shown in Table II. The PAI-1 Ag, tPA-Ag, fibrinogen and TM levels were significantly higher in the masked hypertensive group than in normotensive control group (Table II).

### Discussion

The results of our study have shown that MH is associated with increased plasma levels of PAI, tPA, fibrinogen and TM compared with subjects with normal BP, indicating a decreased fibrinolytic capacity and endothelial damage. This finding indicates that in our study population, MH is associated with a state of decreased fibrinolytic capacity, which may potentially contribute to an increased incidence of cardiovascular events in this group.

The phenomenon of MH is defined as a clinical condition in which a patient's office BP level is  $< 140/90$  mmHg but ambulatory or home BP readings are in the hypertensive range (26). The prevalence of MH in the general population could be as high as 10% (5), whereas data obtained in several cross-sectional studies have demonstrated large differences, with prevalence rates from a low of 8% to a high of 49% (6,27). The prevalence of MH in

our study group was found 19%, which is in the range reported in the general population.

Our results have shown increased levels of fibrinogen in masked hypertensive compared with normotensives. Fibrinogen is a major determinant of blood viscosity and it is also involved in haemostasis/thrombosis pathways. Fibrinogen levels have been shown to be an independent predictor of subsequent cardiovascular events (19,20), underlining the clinical significance of this results.

The impact of MH on TM plasma levels was also put under examination. TM is a protein cofactor expressed on endothelial cells of most blood vessels. Thrombin-bound TM activates protein C, which inhibits thrombin generation by degrading factors Va and VIIIa. TM has also been proposed as a marker of endothelial cell damage and alterations in TM plasma levels have been found to be associated with EH and atherosclerosis (28,29). We found that TM levels are greater in masked hypertensive than in normotensive group. These results are in agreement with the results of a previous study from our clinic showing that subjects with white coat hypertension have increased plasma levels of TM compared with controls. Although the precise mechanisms of TM regulation are not yet quite clear, it has been suggested that hypertensive mediated damage consequently results in endothelial release of this marker (29).

It has been previously shown that essential hypertension is often associated with decreased fibrinolytic potential, procoagulant tendency and endothelial cell damage (15–18), the responsible pathways to this association remain controversial. This has been occasionally attributed to endothelial damage induced by increased BP or to several features of the metabolic syndrome (30,31). It must also be noted that these abnormalities have been observed in normotensive offspring of hypertensive or in hypertensive-prone subjects, indicating the contribution of other factors, unrelated to BP values, including metabolic, neurohumoral and genetic factors as well (32–34). Finally outcome studies have suggested that MH increases cardiovascular risk, which appears to be close to that of in-office and out-of-office (26). These data may provide a plausible explanation for the haemostatic abnormalities observed in masked hypertensives compared with normotensives, although office BP remains in normal values. In conclusion, our study showed that masked hypertensive patients have increased plasma levels of PAI, tPA, fibrinogen and TM compared with normotensives, indicating a procoagulant tendency and endothelial damage to this group.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Table II. Results and comparison between groups.

Parameters	Group A ( <i>n</i> = 24)	Group B ( <i>n</i> = 106)	<i>p</i>
F (mg/dl)	305 $\pm$ 48	260 $\pm$ 30	$<0.01$
TM (mg/dl)	25 $\pm$ 8	17 $\pm$ 10	$<0.01$
PAI-1Ag (IU/ml)	7 $\pm$ 0.6	6.2 $\pm$ 0.6	$<0.001$
tPA-Ag (ng/ml)	8.9 $\pm$ 1.2	7.7 $\pm$ 0.8	$<0.001$
Mean daytime SBP (mmHg)	138 $\pm$ 6	122 $\pm$ 7	$<0.01$
Mean daytime DBP (mmHg)	90 $\pm$ 4	79 $\pm$ 4	$<0.01$

Group A includes patients with masked hypertension and group B normotensive controls. F, fibrinogen; TM, thrombomodulin; PAI-1Ag, plasminogen activator inhibitor-1; tPA-Ag, tissue plasminogen activator.

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