RESEARCH ARTICLE

Clinical application of endothelial injury marker in hypertensive patients

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Purpose: Endothelial damages are one of the most important causes of persistent hypertensive complications. The aim of our study was to discuss the relationship between the molecular markers of endothelial damage, thrombomodulin, and complications in hypertension.

Methods: A total 132 cases of hypertensive patients, including 13 patients with damage of target organs, were selected as research subjects. And grouping was based on different levels of blood pressure. The blood pressure and thrombomodulin levels were detected among all cases, and their drinking, smoking, and other medical records were tracked.

Results: Higher plasma concentration of thrombomodulin was demonstrated in subjects with hypertensive complications compared with without complications [24.5 (18.1,37.55) vs 12.1 (9.1,22.3) TU/mL, $P = .001$, respectively]. The optimum thrombomodulin cutoff value was determined to be more than 15.5 TU/mL, with a sensitivity of 92.3% and a specificity of 63%. With the increase in blood pressure level, thrombomodulin levels in three groups gradually raised [6.15(5.475,12.75) vs 9.75(7.725,13.35) vs 16.45 (10.125,23.725) TU/mL, $P = .007$, respectively].

Conclusion: With the increase in blood pressure and the occurrence of complications, thrombomodulin showed an increasing trend, which was caused by an increase in the degree of endothelial injury. So, thrombomodulin may serve as a clinically meaningful marker of the progression of hypertension.

KEYWORDS

complication, hypertension, thrombomodulin

1 | INTRODUCTION

Hypertension, a common chronic disease, is a leading treatable risk factor for heart, renal disease, and other target organ disease. Kearney et al¹ reported that a quarter of the world's adults were suffering from hypertension and predicted the prevalence of hypertension in the world population would rise to 29.2% in 2025, which is one of the main causes of global burden of disease.² Since 2000, our country has carried out several hypertension investigations. The research indicated that the prevalence of hypertension in China was increasing.³ Patients with hypertension have the characteristics of abnormalities of vessel wall, blood components, and blood flow, which are related

to the damage of target organs and long-term prognosis. Endothelial injury was considered as an early event in the pathophysiology of hypertension⁴ and the basis of many clinical features of hypertension. Therefore, patients with hypertension should be judged by markers of endothelial damage that are readily available in routine clinical practice. Thrombomodulin, a transmembrane glycoprotein, expressed abundantly on the endothelial surface of blood vessels,⁵ first proposed and confirmed by Esmon in 1981, is a high-affinity receptor for thrombin on the surface of endothelial cells. It mediates anticoagulant effect through activation of protein C⁶ and has been implicated in the endothelial regulation of fibrinolysis and coagulation.⁷ It also has the function of maintaining vascular homeostasis and protecting endothelial

cells.⁸ Under normal physiological conditions, the concentration of thrombomodulin in the blood is little;⁹ while the endothelial cells are damaged, thrombomodulin is secreted abnormally and released into the blood,¹⁰ which appears secondary to damage vascular endothelial cells. At present, thrombomodulin has been demonstrated to be a specific biomarker of endothelial cell injury in medicine^{11,12} for the diagnosis and antidiastole of clinical diseases. Thrombomodulin as abnormalities of levels may relate to complications of hypertension and determination of blood pressure itself. The determination of blood levels may contribute to the early diagnosis of subclinical target organ damage. Our aim was to explore the significance of its plasma levels in the pathogenesis of hypertension, by measuring the markers of endothelial injury, thrombomodulin and provide a theoretical basis for its clinical application.

2 | MATERIALS AND METHODS

2.1 | Study population

A retrospective study was performed in 132 patients diagnosed with hypertension during December 2016 and July 2017 at Department of Endocrinology, People's Hospital of Wuhan University. The study included 81 males and 51 females who were a median age of 69.58 years, ranging from 56.05 to 83.11 years. Hypertension was defined as elevated blood pressure ($\geq 40/90$ mm Hg) that was measured on 3 occasions or as treated with antihypertensive agents. Hypertension grading meted the given criteria, Chinese guidelines for the management of hypertension¹³ (as Table 1). Patients with diabetes mellitus, severe pulmonary disease, acute or chronic liver disease, impaired renal function, and peripheral vascular disease were excluded from the study.

2.2 | Blood pressure measurements

Brachial systolic blood pressure and diastolic blood pressure were measured in the right arm with a patient in seated position, assessed by a trained health professional. Thrice evaluations were performed for each patient.

2.3 | Laboratory measurements

Venous blood samples were collected in tubes containing sodium citrate, with anticoagulant ratio of 1:9. The blood samples were centrifuged at 1371 *g*. The level of thrombomodulin was measured by high sensitivity chemiluminescence apparatus, Sysmex HISCL5000 and HISCL thrombomodulin assay kit. The measurement range is 1.0-200 TU/mL, reagent lot number is MK0271, Calibrator ML0191 and general substrate lot number ZS5022. The detection principle is as follows: Biotinylated antithrombomodulin monoclonal antibodies react with thrombomodulin in the sample and bind to streptavidin-coated MP (magnetic particles). After B/F separation, ALP-labeled antithrombomodulin monoclonal antibodies, specifically bind to thrombomodulin on MP. After

TABLE 1 Stage of hypertension

Category	Systolic blood pressure(mm Hg)	Diastolic blood pressure(mm Hg)
Normal	<120	<80
Prehypertension	120-139	80-89
Hypertension	≥ 140	≥ 90
Hypertension-stage1	140-159	90-99
Hypertension-stage2	160-179	100-109
Hypertension-stage3	≥ 180	≥ 110
Isolated systolic hypertension	≥ 140	<90

B/F separation, ALP on MP breaks down CDP-Star Substrate to an excited intermediate, which produces a luminescent signal. Because the light production increases in proportion to thrombomodulin concentration, sample thrombomodulin concentrations can be obtained with a calibration curve prepared with calibrators. Compared with traditional method of ELISA, chemiluminescence rapidly determined automatically the concentration of thrombomodulin in the sample, by counting the photon emission in the enzyme reaction, which has higher sensitivity, shorter detection time, and wider diagnostic range.

2.4 | Statistical analysis

Statistical analysis was performed using SPSS 20.0 software. Differences between the study groups were determined using *t* test or Mann-Whitney U test for independent samples or Kruskal-Wallis H test for multigroup. According to the distribution of the data, the chi-square test was used to calculate the statistical significance for the differences in qualitative parameters. A *P* value of < .5 was taken to be statistically significant.

3 | RESULTS

The general characteristics of study groups are listed in Table 2. A total of 132 cases of hypertensive patients were selected as research subjects, including 13 (9.85%) patients with complications. Among them, the complications of patients with heart diseases consisted of 10 (76.92%) patients, with renal dysfunctions 5 (38.46%) patients, with cerebrovascular diseases 1 (7.69%) patient. Of which 3 cases were complicated with more than two complications. As expected, hypertensions with complications group had significantly higher systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements than without complications did (*P* = .029; .006). However, the characteristics of the subjects, including age, sex, smoking, and drinking history, were the same in the two groups (*P* > .05).

Comparison of thrombomodulin levels in hypertensive patients with or without complications As shown in Figure 1, the concentrations

TABLE 2 Characteristics of hypertensive people with and without complications

	All N = 132	Without complications N = 119	With complications N = 13	P value
Age (y)	69.58 ± 13.53	69.60 ± 13.87	69.46 ± 10.28	.764
Gender (M:F)	81:51	74:45	7:6	.558
SBP (mm Hg)	164.51 ± 28.45	162.83 ± 28.76	183.38 ± 15.98	.029
DBP (mm Hg)	94.15 ± 20.42	92.34 ± 19.29	114.50 ± 23.14	.006
Smoking n (%)	21 (15.91)	19 (15.97)	2 (15.38)	.957
Drinking n (%)	24 (18.18)	21 (17.65)	3 (23.08)	.630

Data are expressed as mean ± SD. F, female; M, male; SBP, systolic blood pressure; DBP, diastolic blood pressure.

of thrombomodulin in hypertensive patients with target organ damage group were higher than that without complications group [24.5 (18.1,37.55) TU/mL vs 12.1 (9.1,22.3) TU/mL, respectively]. The difference was significant ($P = .001$).

The cutoff value with relevant sensitivity and specificity was predicted by ROC analysis. As shown in Figure 2, the area under the curve corresponding to the thrombomodulin proved to be the ideal indicator for anticipating hypertensive complications. The optimum cutoff concentration of thrombomodulin for diagnosing the hypertensive complications was determined to be 15.5 TU/mL. At this level, the sensitivity and specificity were 92.3% and 63%, respectively, with a positive predictive value of 21.4% and negative predictive value of 98.7%.

Comparison of thrombomodulin levels in patients with different grades of hypertension. As shown in Figure 3, with the increase in hypertension grading, the concentration of thrombomodulin was increased [6.15(5.475,12.75) TU/mL vs 9.75(7.725,13.35) TU/mL vs 16.45(10.125,23.725) TU/mL, respectively]. The difference was significant ($P = .007$).

4 | DISCUSSION

With the improvement of people's living standards, China has entered an aging society. The incidence of a variety of chronic diseases

increased year by year. Among them, the incidence of hypertension ranked first in China. It was reported that the prevalence rate of hypertension in adults, over 18 years old, was 18.8% in 2002.¹⁴ In 2006, the number of hypertension patients in China has reached 200 million, which meant that 2 in 10 adults were hypertensive patients averagely.¹⁵ Compared with normotensive subjects, the incidence

FIGURE 2 Area under the curve = 0.793, 95% confidence interval: 0.693-0.894 **FIGURE 1** Without: hypertension without complications; with: hypertension with heart diseases, renal dysfunctions or cerebrovascular diseases. Differences in the hypertensive patients between the two groups were analyzed using Mann-Whitney U test **FIGURE 3** Blood pressure levels of all patients were graded according to guidelines, Chinese guidelines for the management of hypertension. The difference was significant ($P = .007$)

of cardiovascular events in hypertensive patients was significantly increased.^{16,17} Therefore, the treatment and prevention of early hypertension needed to be enhanced. Hypertension is characterized by endothelial damage. The derangement of endothelial function in hypertension may be partly caused by genetic factors, or due to elevated blood pressure itself, due to the relative effects of blood pressure and smooth muscle cells. Vascular sclerosis and peripheral vascular resistance caused by endothelial injury, is a crucial cause of hypertension occurrence and development.¹⁸ As a mechanical barrier for circulating blood and vascular smooth muscle, endothelial impairment is a key mechanism in the pathogenesis of hypertension and its related clinical complications.¹⁹ Previous studies of cultured human umbilical vein endothelial cells showed that the release of thrombomodulin was not secreted by endothelial cells but derived from injured cells.²⁰ There is a positive correlation of thrombomodulin concentration in blood circulation with the degree of injury of endothelial cells.⁶ Thrombomodulin as abnormalities of levels is specific plasma markers of endothelial damage.^{11,12}

We found increased levels of plasma thrombomodulin in patients with target organ damage, compared with without complications ($P = .001$), which demonstrated the endothelial damage in hypertension is closely associated with its complications. Clinically, any screening method used to rule out a disease requires a higher sensitivity and negative predictive value. Our results from the ROC analysis showed that thrombomodulin levels would be a good indicator for the prediction of hypertensive complications. Based on our assay results, a threshold value of 15.5 TU/mL provided 92.3% sensitivity, a negative predictive value of 98.7%, a specificity of 63%, a positive predictive value of 21.4%. The result of our research showed that increase in thrombomodulin was associated with target organ damage in hypertensive patients. Monitoring the change in thrombomodulin may help to determine the target organ damage and its severity. Thrombomodulin levels can increase before complications change and can be used as an early diagnostic marker of target organ damage in hypertensive individuals who are at high risk. Patients with lower levels of thrombomodulin should pay more attention to control blood pressure. Further, in our study, according to the level of blood pressure, patients with hypertension were divided into different grades. The results suggested that the level of thrombomodulin increased with the rise of blood pressure. It indicated that in poorly controlled hypertensive patients, endothelial injury was more noticeable. Dohi Y et al²¹, through cross-sectional studies, indicated increasing levels of plasma thrombomodulin levels in hypertensive patients compared to controlled hypertension. Makris TK²² and Remková A et al¹⁰ reported, as the marker of endothelial injury, thrombomodulin had a higher level in patients with hypertension. Our study results are in agreement with those of some previous reports. Hypertension is a chronic disease. In the course of disease development, we highly recommend to use the 15.5 TU/mL, thrombomodulin levels, for screening hypertensive target organ damage. As this test is more effective than the clinical examination alone, it can be used as a screening test for hypertensive complications.

5 | CONCLUSIONS

In conclusion, elevated levels of thrombomodulin in hypertensive patients reflect early endothelial damage. Measurements of circulating endothelial cell damage marker, thrombomodulin, can be important for improving diagnosis, monitoring, and more effective strategies for the treatment of organ dysfunction in hypertensive patients. Endothelial injury in hypertension is crucial both for the development of the disease process and an important therapeutic target. Detecting the levels of thrombomodulin also becomes crucial.

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