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Visceral Adipose Tissue-Derived Serine Protease Inhibitor (VASPIN) as A Prognostic Marker in Systemic Hypertension

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ABSTRACT

Background: Visceral adipose tissue-derived serine protease inhibitor (VASPIN) is an adipocytokine that exerts anti-migratory and anti-inflammatory roles through certain anti-oxidative effects in the peripheral vascular wall.

Objective: In this study, we studied the relevance of plasma VASPIN levels in patients with hypertension, correlating VASPIN level assay to absence or occurrence of hypertension and its related complications.

Patient and Methods: This was a cross-sectional observational study. A total of 87 subjects were included, divided into 21 age- and sex- matched control (patients' healthy relatives of some of the study- patients) and 66 hypertensive patients. Of the 66 patients with hypertension, thirty-two were newly diagnosed with uncomplicated essential hypertension and thirty four hypertensive patients with macro- and/or microvascular/ complications. Patients with any other chronic diseases like diabetes, chronic renal disease, liver disease, any rheumatological disease and malignancy were excluded. Plasma VASPIN levels and clinical parameters were assessed at baseline for all studied groups.

Results: Comparing VASPIN levels among hypertensive patients showed a negative correlation between serum VASPIN levels and hypertension. Serum VASPIN levels were found lower in the newly diagnosed uncomplicated hypertensive patients group than in control group, and much lower in those with macrovascular and/or microvascular complications group compared to both uncomplicated hypertensive and control groups.

Conclusions: Plasma VASPIN may be used as an independent predictive biomarker for early detection of macrovascular and/or microvascular hypertensive complications.

Keywords: Plasma VASPIN, Biomarker, Hypertension, Microvascular and macrovascular complications.

INTRODUCTION

Adipose tissue is now regarded not only as an energy reservoir, but also as an active endocrine organ, which can secrete a variety of metabolically active adipocytokines [(1)]. VASPIN, a member of the serine protease inhibitor family, is among the adipocytokines secreted from white adipose tissue. VASPIN was first isolated from a rat model of abdominal obesity with type 2 diabetes mellitus (T2DM) [(2)]. Multiple studies showed association between VASPIN concentration and metabolic disorders, including type II diabetes mellitus (T2DM), cardiovascular diseases [(3, 4)], polycystic ovary syndrome [(5)] and osteoarthritis [(6)]. VASPIN was also reported to protect against high fat diet induced bone loss, and to promote osteogenic differentiation [(7)].

VASPIN can exert its anti-inflammatory and anti-migratory roles by a variety of anti-oxidative effects on vascular smooth muscle cells. In this way VASPIN may play an important role in prevention of hypertension [(8)]. **Wang and Wang** [(9)] found that VASPIN levels have an inverse relationship with the risk of developing cardiovascular events, suggesting a protective role of VASPIN in the pathophysiology of coronary atherosclerosis. Although experimental studies have indicated that VASPIN is a vasculo-protective adipocytokine, its specific role and clinical relevance in coronary artery disease (CAD) is not clear [(10)].

Hypertension globally affects individuals in both economically developed and developing nations alike [(11)], and is considered as a major risk for many vascular disorders as heart diseases, strokes (hemorrhagic and ischemic strokes) and chronic kidney disease [(12)].

Recent guidelines have defined hypertension as a sustained systolic blood pressure greater than 130 mm Hg, making almost half of the adult population hypertensive [(13)]. Hypertension may be primary, which may develop as a result of environmental or genetic causes (90-95% of adult cases), or secondary, which has multiple etiologies, including renal, vascular, and endocrine causes (2-10% of cases) [(13)]. The pathogenesis of essential hypertension is multifactorial and complex. Multiple factors modulate the blood pressure (BP) including humoral mediators, vascular reactivity, circulating blood volume, vascular caliber, blood viscosity, cardiac output, blood vessel elasticity, and neural stimulation [(14)]. End-organ damage to the kidneys, heart, brain, and vasculature are among the diverse complications of hypertension. Hence, those who suffer from hypertension are more likely to develop atherosclerosis, stroke, myocardial infarction, heart failure, chronic kidney disease, and dementia [(15)].

Increasingly, it has become apparent that a substantial portion of the vascular, renal, cardiac, and brain damage and dysfunction that accompanies hypertension is mediated by inflammation within these target organs. **Phalitakul *et al.*** [(16)] demonstrated that VASPIN inhibited TNF-α and platelet-derived growth factor (PDGF)-induced inflammatory responses in vascular smooth muscle cells (SMCs) via antioxidant

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mechanisms. Furthermore, **Kameshima *et al.* [8]** found that VASPIN augmented acetylcholine (ACh)-induced endothelium-dependent relaxation via the inhibition of acetylcholine esterase (AChE) in isolated blood vessel, which may suggest that VASPIN has preventive roles on the pathogenesis of hypertension. It was also reported that VASPIN prevents the elevation of systolic blood pressure through inhibition of peripheral arterial hypertrophy possibly via anti-oxidative and anti-inflammatory mechanisms.

In this study, we studied the relevance of plasma VASPIN levels in patients with hypertension and hypertension with complications, correlating VASPIN level assay to absence or occurrence of hypertension and its related complications.

PATIENTS AND METHODS

The study was conducted on 87 subjects, 66 hypertensive patients and 21 healthy control subjects. All patients were recruited from the Outpatient Clinics of Internal Medicine Department at Ain-Shams University Hospital through the period from June 2019 till January 2020.

Ethical approval:

An approval of the study was obtained from Ain Shams University Academic and Ethical committee. Every patient signed an informed written consent for acceptance of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

The sixty-six hypertensive patients were divided into 2 groups: **Group 1** included thirty two newly diagnosed hypertensive patients without complications and **group 2** that included thirty-four essential hypertensive patients with micro and\or macro vascular complications diagnosed by echocardiography, ECG, angiography or fundus examinations,(including ischemic heart disease, hypertensive retinopathy, cerebrovascular stroke, peripheral vascular disase). Twenty-one healthy volunteers (healthy relatives of some of the study patients) were also included in the study as control croup (3).

The diagnosis of hypertension was based on ordinary sphygmomanometer blood pressure measurements according to the international society of hypertension and global hypertension practice gridlines [17]. Office blood pressure (BP) measurement was as follows: Normal BP: <130 mmHg (systolic [SBP]) and < 85 mmHg (diastolic [DBP]). High-normal: 130-139 mmHg SBP and/or 85-89 mmHg DBP. Grade 1 hypertension: 140-159 mmHg SBP and/or 90-99 mmHg DBP. Grade 2 hypertension: ≥160 mmHg SBP and/or ≥100 mmHg DBP. Blood pressure higher than 139/89 on three different readings was the cut off value for diagnosis of hypertension.

Exclusion criteria:

Patients with any other primary chronic diseases like: diabetes or any endocrinal disease, chronic renal disease, chronic liver disease, any rheumatological disease and malignancy.

All subjects were subjected to full history, thorough clinical examination and basic laboratory investigations including CBC, serum uric acid, serum creatinine and urea, serum sodium and potassium levels, liver function tests, TSH, fasting blood sugar (FBS) and HbA1c.

About 10 ml venous blood samples were collected from all patients and control group under aseptic conditions, and serum samples were left to clot at room temperature from 15-20 minutes, then centrifuged for 20 minutes at 2000 – 3000 rpm. Serum uric acid, creatinine, urea, sodium and potassium levels, liver function tests, TSH, fasting blood sugar (FBS) and HbA1c assessment were performed on Cobas Integra, while CBC was done on coulter.

VASPIN levels was assessed for all subjects included in this study where assessment was done using the commercially available ELISA kit (mybiosource, San Diego, California, USA), according to the manufacturer's instruction.

Statistical analysis

Data analysis was performed using the statistical package for special sciences (SPSS) software computer program (version 23.0, IBM Corporation, USA). Data were expressed as mean ± standard deviation (SD) for quantitative data. Whereas, number and percent (%) were used for qualitative data. Independent-samples t test was used when comparing between two groups. One-way analysis of variance (ANOVA) was used when comparing between more than two groups. Post-hoc test (Tukey's) was used to detect the least significant difference (LSD) among the studied groups. Pearson's correlation coefficient (r) test was used for correlating data. Mann-Whitney U test was used to compare quantitative variables, in non-parametric data. Probability (p-value) equal or less than 0.05 was considered significant and less than 0.01 was considered as highly significant.

RESULTS

This study was conducted on 87 subjects divided into three groups. Thirty-two newly diagnosed hypertensive patients (group 1), their ages ranged from 25 to 54 years with a mean of 42.81 ± 6.83 and thirty four hypertensive patients with micro vascular and/or macro vascular complications (group 2), their ages ranged from 34 to 59 years with a mean of 49.38 ± 6.83). Twenty one healthy controls (group 3) with ages that ranged from 34 to 48 years (mean 40.24 ± 4.13) (Table 1).

As regards serum VASPIN levels, in group 1 minimum level was 5 ng/ml, while the maximum level was 85 ng/ml with a mean value of 13.05 ±14.395 ng/ml. In group 2 minimal serum VASPIN level was 2 ng/ml, while the maximum level was 11 ng/ml with a

mean value of 5.57 ± 2.606 ng/ml. Meanwhile, group (3) showed that the minimum serum VASPIN level was 13 ng/ml, while the maximum level was 95 ng/ml with a mean value of 50.14 ± 27.319 ng/ml. Comparing the value of serum VASPIN level between the studied patients' groups and the control group using one way ANOVA test revealed highly statistical significant difference between the studied patients' groups and the control group (P value 0.000*) (Table 2).

On comparison of VASPIN level results in the three groups with each other using post HOC test, there was a significant statistical difference (P value 0.049) on comparing group 1 with group 2. On comparing serum VASPIN level in group 1 with those of group 3 there was a highly significant statistical difference (P value 0.000*). Also comparing serum VASPIN level between group 2 and group 3 showed highly significant statistical difference (P value 0.000*) (Table 3).

Correlation studies of VASPIN serum levels showed a negative correlation between serum VASPIN levels and hypertension progression and complications (Table 2).

Comparing VASPIN level to age among the studied group with Pearson correlation there was a negative correlation between age and serum VASPIN level(- 0.517) (Table 4).,. Comparison of serum VASPIN levels in both gender among the three studied groups showed no statistically significant difference

Table (1): Age of the studied groups

Age (years)	N	Range	Mean	Std. Deviation
Hypertensive patients (Group 1)	32	25-54	42.81	6.83
Hypertensive patients complicated patients (Group 2)	34	34 -59	49.38	6.83
Control group (Group 3)	21	34 - 48	40.24	4.13

Table (2): Comparison of VASPIN level between the three studied groups

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	F test	P
				Lower Bound	Upper Bound				
Hypertensive patients	32	13.05	14.395	7.86	18.24	5	85	53.790	.000*
Hypertensive patients complicated patients	34	5.57	2.606	4.66	6.48	2	11		
Control group	21	50.14	27.319	37.71	62.58	13	95		
Total	87	19.08	23.919	13.98	24.18				

Table (3): Post hoc tests (to compare between the studied groups)

Groups		P
Hypertensive patients	Control group	0.000*
	Hypertensive patients complicated patients	0.049
Hypertensive patients complicated patients	Control group	0.000*
	Hypertensive patients	0.049
Control group	Hypertensive patients	0.000*
	Hypertensive patients complicated patients	0.000*

Table (4): Correlation between age and VASPIN in the whole sample

		VASPIN
Age	Pearson Correlation	-0.517**
	Sig. (2-tailed)	0.001
	N	**87**

DISCUSSION

Hypertension is the commonest disease affecting people health worldwide and is an important risk factor of many common health problems that endanger human wellbeing as heart diseases, strokes as well as other vascular diseases, and chronic kidney diseases. The exact etiology of essential hypertension that constitutes more than 90% of cases of hypertension is still unclarified [(18)].

VASPIN, a serine protease inhibitor secreted from white adipose tissue, has been shown to have an anti-inflammatory effect, anti-apoptotic effect, as well as protective effects on vascular walls as shown in studies on experimental rat models [(19)]. Many studies discussed the role of VASPIN in atherosclerosis and coronary artery diseases, while fewer studies investigated the role of VASPIN in hypertension and its complications.

In the present study, patients with hypertension, both uncomplicated and those with macrovascular and/or microvascular complications, were shown to have significantly lower VASPIN levels compared to healthy controls ($p<0.000$). Moreover, hypertensive patients with macrovascular and/or microvascular complications (group 2) exhibited significantly lower VASPIN levels when compared to hypertensive patients without complication (group 1) ($p <0.000^*$). These results are in agreement with previous studies who reported a significant correlation between plasma VASPIN concentrations and the presence of atherosclerosis, which is considered to be one of the major elements in the pathogenesis of hypertension, and severity of coronary artery stenosis and atherosclerosis [(20, 21, and 22)]. Similarly, **Zhang *et al.*** [(23)] reported that low VASPIN level is correlated with the severity of coronary artery disease, which supports our findings that hypertensive patients with higher VASPIN levels exhibits less complications. On the other hand, **Rueda-Gotor *et al.*** [(24)] found that there was no statistically significant association between VASPIN levels and markers of atherosclerosis.

In our study, we found a negative correlation between VASPIN levels in hypertensive patients compared to the control group, which may signify that serum VASPIN levels may have a protective role against developing hypertension. Similarly, **Dimova and Tankova** [(10)] suggested that VASPIN level could be used as a predictor for major events as coronary artery disease and carotid artery stenosis. In that context, **Lin and his associates** [(25)] observed that VASPIN may have an important role in protection against the progression of atherosclerosis, and suggested that VASPIN may be useful for preventing vascular diseases. Moreover, **Ji *et al.*** [(26)] stated that plasma VASPIN levels were found to be an independent predictor of major adverse cardiac events in patients with chest pain. The exact mechanism by which VASPIN protect against atherosclerosis, hypertension and their complication is not fully understood. **Kameshima *et al.*** [(27)] reported that VASPIN prevents elevation of blood pressure through inhibition of peripheral vascular remodeling in spontaneously hypertensive rats, through inhibiting peripheral vascular hypertrophy possibly via anti-oxidative and anti-inflammatory mechanisms. VASPIN could also exhibit its protective effect against hypertension through inhibition of TNF-α and platelet-derived growth factor (PDGF)-induced inflammatory responses in vascular smooth muscle cells (SMCs) via antioxidant mechanisms as reported by **Venegas *et al.*** [(28)] and **Phalitakul *et al.*** [(16)], or through inhibition of acetylcholine esterase (AChE) in isolated blood vessel so induce blood vessels endothelium relaxation as reported by **Kameshima *et al.*** [(27)].

CONCLUSION

According to this study serum VASPIN levels may be a useful independent biomarker for predicting and searching for hidden or impending macro/micro-vascular complications in hypertensive patients specially, uncontrolled hypertensive ones.

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