NOTE

Progression of Diabetic Nephropathy Enhances the Plasma Osteopontin Level in Type 2 Diabetic Patients

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Abstract. Osteopontin (OPN) is thought to play multiple roles in the progression of atherosclerotic plaque including diabetic vascular complications. However, it still remains unclear whether the level of OPN in vivo is indeed clinically associated with the progression of diabetic complications. This study evaluated whether the levels of OPN in plasma and urine are correlated with the progression of diabetic complications, such as retinopathy, neuropathy, and nephropathy in patients with type 2 diabetes. In 229 patients with type 2 diabetes, OPN level in plasma and urine was evaluated by both the severity of diabetic complications, such as retinopathy, neuropathy, and nephropathy, and the clinical characteristics and the substantial laboratory findings. Plasma OPN level increased significantly with aging and the progression of diabetic nephropathy, especially at the stage of renal failure (p<0.05). However, the level was not related to the progression of retinopathy or neuropathy, or to laboratory findings, such as HbA1c or serum lipids. In contrast, urinary OPN level was not associated with diabetic complications in any of the subjects. There was no correlation between the plasma and urinary values of OPN. The results established that the plasma OPN was elevated in proportion to the progression of diabetic nephropathy, indicating that the plasma concentration may be a potential diagnostic predictor of diabetic end-stage renal disease.

Key words: Osteopontin, Diabetes mellitus, Diabetic nephropathy, Aging


OSTEOPONTIN (OPN) is a multifunctional glycoprotein secreted from various cell types, such as osteoclasts, lymphocytes, macrophages, epithelial cells and vascular smooth muscle cells (VSMCs), which binds calcium and contains an arginine-glycine-aspartate motif for interaction with the integrin family of cell adhesion molecules [1]. OPN is chemotactic and acts as an extracellular matrix protein and a cytokine and a growth factor [2]. Although OPN is not present in most normal soft tissues, it is highly localized to the surfaces of calcified deposits in certain pathological conditions [3–6], suggesting that OPN may be an important regulator of ectopic calcification.

In addition, several investigators have recently revealed that OPN can play multiple roles in the progression of atherosclerotic plaque [7–14] including diabetic vascular complications [12–14]. Particularly in diabetes, OPN has the potential to promote both the growth and migration of vascular wall cells such as VSMCs [9, 10, 12, 14] and mesangial cells [13], depending on the glucose concentration. This implies that OPN can directly contribute not only to the tissue calcification process but also to the development of diabetic vascular complications. However, these studies have evaluated the local expression of both protein and mRNA of OPN in diabetic animal tissues including neointima as well as atheromatous plaques or the changes of the intracellular response in cultured
cells by treatment with OPN. It still remains unclear whether the OPN level in vivo is indeed clinically associated with the progression of diabetic complications.

Therefore, in the present study, we attempted to determine whether the levels of OPN in plasma and urine were correlated with the progression of diabetic complications, such as retinopathy, neuropathy, and nephropathy, in type 2 diabetic patients.

**Research Design and Methods**

A total of 229 patients with type 2 diabetes were recruited from the outpatient clinic at Yamagata University Hospital. Height and body weight were measured to calculate body mass index (BMI). Systolic and diastolic blood pressure was also determined using a sphygmomanometer. Laboratory samples were obtained in the morning, and information was collected concerning the level of plasma glucose and HbA1c, the serum levels of total cholesterol (T-Cho), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C), and the urinary level of albumin and creatinine (Cr). OPN levels in both plasma and urine samples were measured using a solid-phase sandwich enzyme-linked immunosorbent assay kit (Immuno-Biological Laboratories, Gumma, Japan), as described previously [15]. In urine samples, to remove the influence of the urinary volume, the values were normalized by the urinary Cr level.

Each stage of diabetic complications in the patients (retinopathy, neuropathy, or nephropathy) was defined as described previously [16, 17]. The stage of retinopathy was divided into four groups and evaluated by ophthalmologists from Yamagata University Hospital (Normal, no finding of retinopathy; Mild, defined by the presence of microaneurysms plus mild-to-moderate retinal hemorrhage or hard exudates; Moderate, defined by the presence of microaneurysms plus any cotton-wool spots, mild intraretinal microvascular abnormalities, venous beading, or severe retinal hemorrhage; Proliferative diabetic retinopathy (PDR), defined by the growth of new blood vessels and posterior surface of the vitreous). The stage of nephropathy was also divided into four groups by the urinary findings and serum Cr level (Normal, urinary albumin excretion rates (AER) <30 μg/mg Cr; Microalbuminuria, 30 μg/mg Cr≤AER; Renal failure, serum Cr level ≥2 mg/dl). The presence of neuropathy was defined by physical examination plus either abnormal nerve conduction velocity in two different peripheral nerves or unequivocally abnormal autonomic results.

Results were expressed as the mean ± SD. Statistical significance was estimated by linear regression analyses for the correlation of the parameters and the Mann-Whitney U test, and the difference was considered to be significant at p<0.05.

**Results**

As shown in Table 1, the OPN levels in both plasma and urine were 395.6 ± 172.9 ng/ml and 30.5 ± 22.3 ng/g Cr, respectively. There was no relationship between the plasma and the urinary values of OPN. To characterize the clinical identity of OPN in diabetic patients, we first examined the relationships among the OPN values in plasma and urine and the characteristics of several potential parameters, which included age, gender, BMI, duration of diabetes, and values of blood pressures, HbA1c, and serum lipids. The plasma OPN value was, though small, significantly correlated with only age (r = 0.152, p<0.05) (Fig. 1), but not with other parameters. In contrast, the urinary OPN value was not related to any parameters. In addition, not even the difference in the type of treatment for diabetes affected the plasma or the urinary OPN value.

Next, we examined whether the OPN level in plas-
ma or urine is elevated in proportion to the progression of diabetic complications. As shown in Fig. 2, there was no change in the plasma OPN level by the progression of diabetic retinopathy or neuropathy. In contrast, interestingly, in diabetic nephropathy, compared with the normal group, the plasma OPN level increased at the stage of macroalbuminuria and reached 688.5 ± 241.0 ng/ml at the stage of renal failure (p<0.05). The plasma OPN levels of two participants, one in the stage of macroalbuminuria and the other in the stage of renal failure, increased to above 1000 ng/ml. In addition, although we examined the progression of diabetic complications and whether they could affect the urinary OPN level, no change was observed.

**Discussion**

Extensive studies have recently clarified that OPN is widespread and localized in several pathological organs and has important roles in ectopic calcification [6, 8], wound healing [4], several types of cancer [15, 18], and vascular remodeling, including diabetic condition [7–14], granulomatous inflammation [3], and glomerulonephritis [5, 19]. Although the clinical implications of these findings have yet to be fully clarified, it is more likely that OPN can, at least, evolve as a major player in the injury/repair cascades. In addition, considerable evidence supports the view that the enhancement of OPN in diabetic vasculature might also be related to the development of vascular complications since several investigators have shown that high glucose can stimulate OPN expression in the cultured vascular wall cells, leading to exaggerated cell growth [12, 13]. Recent reports have suggested that, in streptozotocin-induced diabetic rats, the OPN level was more elevated in the medial layer of carotid arteries [13] and the tubular epithelium of renal cortex [20] than in the control.

In this study, we could show that the plasma OPN level increased with the progression of nephropathy, but not retinopathy or neuropathy, in diabetic patients (Fig. 2). As severe end-stage renal disease (ESRD) is one of the most crucial risk factors for mortality and
morbidity in diabetic patients [21, 22], it seems likely that the plasma OPN level may be useful as one of the potential biomarkers for the severity of nephropathy. In agreement with our findings, Moe et al. have recently reported that, in patients with ESRD, positive immunostaining for OPN in the artery is stronger in diabetes than in non-diabetes [23]. Remarkably, the plasma OPN levels of two participants were above 1,000 ng/ml. Although one patient at the stage of renal failure, whose plasma OPN levels reached 1,069 ng/ml, did not have retinopathy, treatment with hemodialysis was performed 3 months after the measurement. In contrast, another participant at the stage of macroalbuminuria, whose plasma OPN levels reached 1,372 ng/ml, had been histologically diagnosed with diabetic nephropathy with membranous nephritis by renal biopsy. Since several investigators have recently shown that OPN could participate in the formation and progression of autoimmune and experimental nephritis [24–27], it is plausible to assume that a patient with an extremely high plasma OPN level, but not at the stage of renal failure, should be advised to have a renal biopsy to diagnose additional nephritis.

Although the reason why the plasma OPN level was elevated only in the progression of nephropathy but not in that of retinopathy remains unclear, it is reasonable to assume that the plasma OPN level in the whole body might result from the size of each organ itself, and that the OPN level in the retinal tissue could be locally increased. Takagi et al. have recently shown that the expression of OPN is increased in the ischemic retina and contributes to vascular endothelial proliferation and subsequent retinal neovascularization in diabetic mice [28]. In contrast, although the mechanism that prevents the plasma OPN level from becoming significantly elevated during the progression of neuropathy is still unknown, one possibility is the difficulty of evaluating neuronal damage in diabetic patients by quantitative analysis. In fact, Chabas et al. have revealed that the transcription for OPN is enhanced by the demyelination in the brain from patients with multiple sclerosis [29], it is likely that the plasma OPN level might be more elevated in patients in the severe stages of neuropathy.

Although we have evaluated the relationship with the plasma OPN value and some reliable parameters, the association was only found with aging and not even in the value of HbA1c or serum lipids. Several investigators have reported that the elevated expression of OPN in vasculature has a crucial role in atherosclerotic calcification [6, 8, 23, 30]; however, the significance of whether the enhancement is inducible or preventive to the vascular calcification has not been fully revealed. In addition, recent studies have shown that, in vascular wall cells, the expression of OPN is accelerated by advanced glycation end-products [31] and mechanical stress [32]. Therefore, it might be considered that these findings can be associated with the mechanism(s) of elevating the plasma OPN level by both aging (Fig. 1) and the progression of nephropathy (Fig. 2). In contrast, Takemoto et al. have revealed that both LDL-C and a statin can reduce the expression of OPN in VSMCs [33]. In this study, although the precise mechanism whereby the value of OPN was not related to those of HbA1c or serum lipids is still obscure, it is likely that the difference may result from the effects of some agents for diabetes and dyslipidemia. In fact, there was no change on the plasma OPN level with or without statin (398.6 ± 27.8 ng/ml vs. 394.6 ± 12.2 ng/ml). Furthermore, although we examined whether the plasma OPN level in participants without statin treatment could be influenced by the progression of diabetic complications, the level was increased in proportion to the progression of diabetic nephropathy alone and significantly different at the stage of renal failure compared with that at the stage of normoalbuminuria (data not shown).

In conclusion, the results of this study have established that the plasma OPN level was elevated as a result of the progression of diabetic nephropathy, which suggests that the plasma concentration of OPN may be a potential diagnostic biomarker for the prediction of diabetic ESRD.

References


