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Association of plasma osteopontin with diabetic retinopathy in Asians with type 2 diabetes

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Purpose: Osteopontin (OPN) is a proinflammatory cytokine with diverse functions. Increased levels of OPN in vitreous fluid have been reported in patients with diabetic retinopathy (DR); however, studies on circulating OPN levels in DR are limited. We aim to examine the association of plasma OPN levels with the presence and severity of DR in a multiethnic cohort with type 2 diabetes mellitus (type 2 diabetes) in Singapore.

Methods: Plasma levels of OPN were measured using enzyme-linked immunosorbent assay. Digital color fundus photographs were assessed for DR. DR severity was categorized into non-proliferative DR (NPDR) and proliferative DR (PDR). Gradable fundus photographs and OPN measurements for 443 patients were used for analysis. A logistic regression model was used to evaluate the association of OPN with DR.

Results: DR was diagnosed in 174 (39.3%) patients, including 132 (75.9%) with NPDR and 42 (24.1%) with PDR. The median of OPN was higher in the patients with DR (64.7 [49.7–89.5] ng/ml) than in the patients without DR (51.7 [38.9–66.9] ng/ml; p<0.001). After adjustment for clinical and biochemical factors, a 1-unit increase in nature logarithm (ln)-transformed OPN was associated with the presence of DR (2.770 [1.599–3.800], p<0.001). The area under the curve (AUC) increased statistically significantly after the addition of OPN (0.805[0.763–0.846] versus 0.825 [0.785–0.865], p=0.011). In the severity analyses, the median of OPN was statistically significantly higher in the patients with PDR (76.8 [55.0–103.6] ng/ml) than in the patients with NPDR (61.7 [47.7–87.3] ng/ml; p=0.017). After adjustment, the 1-unit increase in lnOPN remained associated with NPDR (2.673 [1.519–4.704], p=0.001) and PDR (3.389 [1.254–9.226], p=0.017), respectively (p-trend=0.001).

Conclusions: Plasma OPN levels were associated with the presence and severity of DR in patients with type 2 diabetes, suggesting OPN may be useful as a potential biomarker for DR.

Type 2 diabetes mellitus (type 2 diabetes) is a rapidly evolving global health issue, and Asia is the epicenter of this global epidemic [1]. In Singapore, the prevalence of type 2 diabetes has been predicted to double from 7.3% in 1990 to 15% in 2050 [2]. Diabetic retinopathy (DR) is one of the most common microvascular complications of type 2 diabetes and is a leading cause of irreversible blindness among adults of working age [3]. Although the prevalence of DR in Asians (12.1% to 23.0%) is generally lower than in Western populations (28.5% to 43.5%), Singapore has higher prevalence of DR (25.4% to 35.0%) than other Asian countries, reaching close approximation with Western countries [4].

Osteopontin (OPN), also known as secreted phosphoprotein 1 (SPP1), is a multifunctional glycoprotein that is expressed by various cell types and exists as an immobilized extracellular matrix protein and as a soluble proinflammatory cytokine [5]. This hyperglycemia-induced cytokine exhibits diverse functions, such as angiogenesis, inflammation, and fibrosis [5], the key pathogenesis processes involved in vascular complication of diabetes [6]. An experimental study has demonstrated upregulation of OPN in retinal endothelial cells under a high glucose environment, which may induce endothelial cell proliferation and retinal neovascularization [7]. These studies suggest that OPN may play an important role in the etiopathogenesis of DR [7].

However, to date, there is a paucity of studies that have investigated the role of OPN in DR. Three studies with small sample sizes measured OPN levels in the vitreous fluid, and data from these studies consistently demonstrated higher levels of OPN in patients with any type of DR, as well as PDR, when compared to diabetic patients without DR [8-10]. Of note, measurement of OPN levels in vitreous fluid may have limited clinical application because vitreous fluid can be obtained only invasively in a subgroup of patients with DR undergoing surgery as part of their clinical management (i.e., non-resolving vitreous hemorrhage and tractional retinal detachment). OPN levels in easily and inexpensively collected

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samples, such as plasma, may represent a valuable source for evaluating OPN-related risks and a potential screening tool for DR. To date, only one study has measured plasma levels of OPN in patients with DR and found a positive but non-statistically significant relationship between plasma OPN levels and DR [11]. Moreover, all previous studies examined the association of OPN and DR only in univariate analysis without taking into account the effect of confounding factors, such as duration of diabetes and blood pressure [12]. To our knowledge, the role of circulating levels of OPN in DR has never been reported in a Singaporean population. In this study, we aim to evaluate the association of plasma OPN levels with the presence and severity of DR in a multiethnic Asian cohort with type 2 diabetes in Singapore.

METHODS

Study population and design: We included 482 subjects with available digital color fundus photographs from the Singapore Study of Macroangiopathy and Microvascular Reactivity in Type 2 Diabetes (SMART2D), a cross-sectional study of 2,057 adults aged 21–90 years with type 2 diabetes that was conducted between August 2011 and February 2014. Inclusion and exclusion criteria of SMART2D have been previously described [13]. Generally, these 482 subjects have a similar profile as unselected subjects in SMART2D (Appendix 1). Thirty-nine subjects were not included due to the following reasons: type 1 diabetes (n=3), missing clinical information (n=1) or OPN measurement (n=21), and non-gradable photos (n=14). Finally, 443 subjects were included in the analysis. This study was approved by the National Healthcare Group Domain Specific Review Board (NHG-DSRB). This study adhered to the tenets of the Declaration of Helsinki and the Association for Research in Vision and Ophthalmology (ARVO) statement on human subjects. Individual written informed consent was obtained in all subjects before enrollment in the study.

Measurement of plasma levels of OPN: Plasma levels of OPN were measured using the enzyme-linked immunosorbent assay (ELISA) kit Quantikine Human Osteopontin Immunoassay (R&D Systems Inc., City, Minneapolis, MN) according to the manufacturer's instructions. In brief, serum samples were diluted 1:25 with calibrator diluent. A twofold serial dilution of a manufacturer-provided OPN standard was included; the 20 ng/ml standard was the high standard, and a calibrator diluent was the sample blank (0 ng/ml). A microplate reader (Bio-Rad, Irvine, CA) was used to quantify the signal at 450 nm. The intra- and inter-assay coefficients of variation were 2.6–4.0% and 5.4–6.6%, respectively. The sensitivity reported by the manufacturer was 0.011 ng/ml.

Assessment of DR: Non-mydriatic digital images of the retina for both eyes were taken in all study subjects using a retinal camera (TRC-NW 200, Topcon Co., Tokyo, Japan). Digital color fundus photographs were assessed for the presence of DR by a fellowship-trained retina specialist in a masked fashion to minimize any possible bias. The photographs were not graded and were labeled as non-gradable if more that 50% of the retinal photographs were not clearly visible.

DR was considered present if any characteristic lesions as defined by the Early Treatment Diabetic Retinopathy Study were present. The minimum criterion for diagnoses of DR was the presence of at least one definite microaneurysm and/or retinal hemorrhage. DR severity was further categorized into non-proliferative DR (NPDR) and proliferative DR (PDR) [14]. DR was classified as NPDR based on the presence of one or more of the following features: microaneurysms, hemorrhages, hard or soft exudates, venous beading, and intraretinal microvascular abnormalities. DR was classified as PDR if there was neovascularization, preretinal hemorrhages, vitreous hemorrhage, or panretinal laser photocoagulation scars.

Clinical and biochemical measurement: Blood pressure (BP), urinary albumin-to-creatinine ratio (ACR), estimated glomerular filtration rate (eGFR), hemoglobin A1c (HbA1c), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), total triglycerides, and a soluble form of advanced glycation end products (sRAGE) were measured as described previously [13]. Plasma levels of pigment epithelium-derived factor (PEDF) were measured and quantified with ELISA (Biovendor Laboratory Medicine, Modrice, Czech Republic).

Statistical analysis: Normally distributed continuous data were expressed as means and standard deviations. Skewed variables were expressed as median and inter-quartile range (IQR) and nature logarithm (ln)-transformed before data analysis. For normally distributed continuous data, the *t* test was used to compare means. For skewed continuous data, the Wilcoxon rank-sum test was used to compare medians. A chi-square test was used to compare the distributions of categorical variables.

Binary logistic regression was used to examine the association of OPN level with the presence of DR. Variables that were statistically significant in univariate analysis (Appendix 2) or with putative roles in the pathobiology of DR were added for adjustment. Performance in prediction of the presence of DR was assessed with the area under the curve (AUC) calculated before and after the addition of OPN levels into the model based on non-parametric approaches [15]. Multinomial logistic regression was used to examine the association of

OPN level with the severity of DR. Ordinal logistic regression models were used to estimate the overall trend of these associations. All statistical analyses were performed using STATA version 14.0 (STATA Corporation, College Station, TX). A two-tailed p value of less than 0.05 was considered statistically significant.

RESULTS

DR was found in 174 of the 443 (39.3%) patients, including 132 (75.9%) patients with NPDR and 42 (24.1%) patients with PDR. Table 1 summarizes the characteristics of patients with type 2 diabetes stratified by the presence of DR. The patients with DR were older and had a longer duration of diabetes, higher systolic BP, diastolic BP, HbA1c, ACR, and sRAGE, and lower eGFR compared with the patients without DR. The percentage of current and former smokers, neuropathy, and commonly used medications in diabetes (i.e., insulin and/or oral hypoglycemic medications) was statistically significantly higher in patients with DR than in patients without DR. OPN levels were statistically significantly higher in patients with DR (median (IQR): 64.7 (49.7–89.5) ng/ml) than in patients without DR (51.7 (38.9–66.9) ng/ml; p<0.001). Patients with DR also had a statistically significantly higher level of sRAGE (850.0 (552.8–1216.2) versus 726.6 (525.9–1054.7) pg/ml, p<0.001) and PEDF (16.5 (13.5–20.1) versus 15.1 (12.4–18.3) ng/ml, p=0.006) than patients without DR.

In univariate analysis, a 1-unit increase in lnOPN levels was associated with the presence of DR (odds ratio (OR)=3.442, 95% confidence interval (CI), 2.218–5.342, p<0.001; Appendix 2). The association remained statistically significant in the multivariate logistic regression model (OR=2.770, 95% CI, 1.599–3.800, p<0.001) adjusted for age, gender, ethnicity, type 2 diabetes duration, HbA1c, smoking, systolic BP, diastolic BP, eGFR, sRAGE, PEDF, and commonly used medications in diabetes (Table 2).

The median of the OPN levels was statistically significantly higher in patients with PDR (76.8 (55.00–103.6) ng/ml) when compared with patients with NPDR (61.7 (47.7–87.3) ng/ml; p=0.017; Figure 1). Table 2 shows the association of OPN with the severity of DR using the multinomial logistic regression model. With a 1-unit increase in lnOPN, the OR for patients with NPDR and PDR was 2.673 (95% CI, 1.519–4.704, p=0.001) and 3.389 (95% CI, 1.245–9.226, p=0.017), respectively (the linear trend of the OR, p-trend=0.001).

Figure 2 shows the predictive ability of OPN alone, the variables (clinical and biochemical factors) adjusted in the model, and the combination of OPN plus variables. The AUC was 0.679 (95% CI, 0.627–0.730) for OPN and 0.805 (95% CI, 0.763–0.846) for the combined variables. When OPN was added to the model consisting of the variables above, there was a statistically significant improvement in the AUC (0.825, 95% CI, 0.785–0.865, p=0.011).

DISCUSSION

This is the first study to demonstrate that plasma OPN levels are positively and statistically significantly associated with the presence and severity of DR. Furthermore, there was a statistically significant improvement in the risk prediction for DR when OPN was incorporated in the risk prediction model with known clinical and biochemical risk factors for DR.

Previous studies have demonstrated higher levels of OPN in the vitreous fluid from DR than those in diabetes patients without DR [8-10]. Because vitreous fluid is highly inert and protected by the blood–retinal barrier, it is postulated that the elevated OPN level is not due to the breakdown of the blood–retinal barrier but can be expressed in local intraocular tissues (i.e., ganglion cells in retinal tissue) and secreted into the vitreous fluid under diabetic conditions [16]. Although OPN in vitreous fluid may represent an organ-specific biomarker, it can be obtained only invasively in patients undergoing surgery, thus limiting the clinical importance. Under pathological conditions, high plasma levels of OPN may represent cumulative levels of OPN produced by multiple organs, such as bone and kidneys [17]. We believe that the higher levels of OPN observed in this study may be attributable to the involvement of multiple organs secondary to the diabetic disease process, and therefore, may represent the total disease burden, including eye disease.

To our knowledge, only one study has measured plasma levels of OPN in patients with type 2 diabetes [11]. Although this Japanese study demonstrated higher plasma levels of OPN in patients with NPDR (n=82) and PDR (n=18) when compared to patients without DR (n=129), the relationship did not reach statistical significance probably due to the small sample size. In this study with a larger sample size, we observed statistically significant higher plasma levels of OPN in patients with NPDR and patients with PDR than in patients without DR. Although both studies measured plasma OPN levels using solid-phase ELISA, the plasma levels of OPN were higher in the Japanese population than in the population in the present study. It may be related to methodological differences between the ELISA kits, for instance, antibody targeting sites that may actually target different molecular forms of OPN present in vivo. Alternatively, it may be attributable to differences between the Japanese population and the present study population in terms of age (63.0 versus 58.5 years), duration of diabetes (14.0 versus 11.2 years), and body-mass index (BMI; 24.0 versus 28.3 kg/m^2). In the present

Table 1. Clinical and biochemical characteristics of individuals with T2DM stratified by presence of DR (n=443).

Variables	DR (174)	Non-DR (269)	All (443)	P-value
Entry age (yrs)	55.3±9.2	51.4±12.5	53.0±11.5	**<0.001**[a]
Male gender (%)	57.3	59.3	58.5	0.085[b]
Ethnicity (%)				
Chinese	45.4	62.1	55.4	
Malays	27.3	14.5	19.6	
Indians	27.3	23.4	24.9	**0.001**[b]
Smoking (current and former; %)	27.1	16.6	20.8	**0.007**[b]
T2DM burden				
Duration (yrs)	14.0±9.0	9.3±8.2	11.2±8.9	**<0.001**[a]
HbA1c (%)	8.4±1.4	7.9±1.5	8.1±1.5	**<0.001**[a]
Oral glycemic medication[1] (%)	90.3	91.5	91.0	0.170[b]
Insulin (%)	51.7	29.6	38.3	**<0.001**[b]
Oral glycemic medication & insulin (%)	43.5	26.2	33.0	**<0.001**[b]
RAS medication[2] (%)	76.4	56.5	64.3	**<0.001**[b]
History of CVD				
IHD[3] (%)	13.3	11.5	12.3	0.192[b]
Stroke (%)	7.5	3.0	5.0	0.150[b]
Neuropathy[4] (%)	27.3	3.9	13.2	**<0.001**[b]
SBP (mmHg)	148.4±19.3	136.5±17.4	141.2±19.0	**<0.001**[a]
DBP (mmHg)	82.5±10.4	80.5±10.1	81.3±10.3	**0.046**[a]
BMI (kg/m^2)	28.4±5.7	28.2±5.5	28.3±5.6	0.742[a]
HDL-C (mM)	1.27±0.31	1.25±0.34	1.26±0.33	0.591[a]
LDL-C (mM)	2.82±0.81	2.80±0.76	2.81±0.78	0.821[a]
eGFR (ml/min/1.73 m^2)	78.9 (51.7 −104.7)	93.8 (76.2–120.6)	87.3 (68.7–113.5)	**<0.001**[c]
sRAGE (pg/ml)	850.0 (552.8–1216.2)	726.6 (525.9–1054.7)	768.4 (538.7–1115.6)	**0.037**[c]
PEDF (ng/ml)	16.5 (13.5–20.1)	15.1 (12.4–18.3)	15.6 (12.7–18.9)	**0.006**[c]
OPN (ng/ml)	64.7 (49.7–89.5)	51.7 (38.9–66.9)	56.2 (43.9–74.0)	**<0.001**[c]

[1]Usage of insulin secretagogues, Rosi-/Pio-glitazone or metformin;[2]Renin-angiotensin system (RAS) medication, angiotensin-converting-enzyme or angiotensin receptor blockers; [3]Ischemic heart disease (IHD), blockade of arteries to the heart, heart Attack, balloon angioplasty of blocked artery of the heart, or heart bypass operation; [4]Neurothesiometer reading>25 V or monofilament sensory test result below 8 out of 10 points on either side of the feet. HbA1C, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; sRAGE, soluble receptor for advanced glycation end products; sRAGE: soluble receptor for advanced glycation end-products; PEDF, pigment epithelium-derived factor; OPN, osteopontin [a]*t* test, [b]χ^2 test, [c]Wilcoxon rank-sum test

Table 2. Association of OPN and the presence and severity of DR in adjusted model (n=443).

Variables	Presence of DR		Severity of DR			
	DR (174)		NPDR (132)		PDR (42)	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Entry age (yrs)	0.990 (0.964–1.018)	0.496	1.004 (0.975–1.033)	0.808	0.928 (0.880–0.977)	0.056
Male gender	0.694 (0.401–1.995)	0.191	0.816 (0.458–1.453)	0.489	0.675 (0.255–1.783)	0.428
Ethnicity						
Chinese	Ref.					
Malay	2.394 (1.265–4.532)	**0.007**	2.543 (1.316–4.916)	**0.005**	1.540 (0.514–4.607)	0.440
Indian	2.913 (1.610–5.271)	**<0.001**	2.862 (1.537–5.331)	**0.001**	3.514 (1.253–9.853)	**0.017**
T2DM duration (yrs)	1.017 (0.985–1.049)	0.294	1.001 (0.968–1.035)	0.946	1.100 (1.044–1.160)	**<0.001**
HbA1c (%)	1.344 (1.116–1.618)	**0.002**	1.362 (1.122–1.652)	**0.002**	1.263 (0.914–1.747)	0.157
Smoking (current and former)	2.418 (1.268–4.611)	**0.007**	2.687 (1.386–5.208)	**0.003**	1.165 (0.348–3.897)	0.804
SBP (mmHg)	1.037 (1.018–1.057)	**0.001**	1.032 (1.012–1.052)	**0.002**	1.052 (1.021–1.084)	**0.001**
DBP (mmHg)	0.992 (0.961–1.025)	0.647	0.998 (0.965–1.032)	0.914	0.962 (0.913–1.012)	0.138
BMI (kg/m^2)	0.976 (0.929–1.025)	0.334	0.984 (0.934–1.036)	0.533	0.939 (0.859–1.025)	0.161
LneGFR (ml/min/1.73 m^2)	0.284 (0.145–0.553)	**<0.001**	0.335 (0.165–0.680)	**0.002**	0.172 (0.067–0.439)	**<0.001**
RAS medication	1.717 (0.993–2.970)	0.053	1.727 (0.971–2.926)	0.063	1.724 (0.612–4.864)	0.303
Oral glycemic medication & Insulin	1.424 (0.806–2.515)	0.224	1.484 (0.816–2.698)	0.195	1.190 (0.477–2.969)	0.709
LnsRAGE (pg/ml)	1.303 (0.817–2.078)	0.267	1.151 (0.705–1.878)	0.573	2.652 (1.139–6.175)	**0.024**
LnPEDF (ng/ml)	0.392 (0.154–0.996)	0.051	0.361 (0.135–0.963)	**0.042**	0.618 (0.116–3.285)	0.572
LnOPN (ng/ml)	2.770 (1.599–3.800)	**<0.001**	2.673 (1.519–4.704)	**0.001**	3.389 (1.245–9.226)	**0.017**

HbA1C, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; sRAGE, soluble receptor for advanced glycation end products; sRAGE: soluble receptor for advanced glycation end-products; PEDF, pigment epithelium-derived factor; OPN, osteopontin

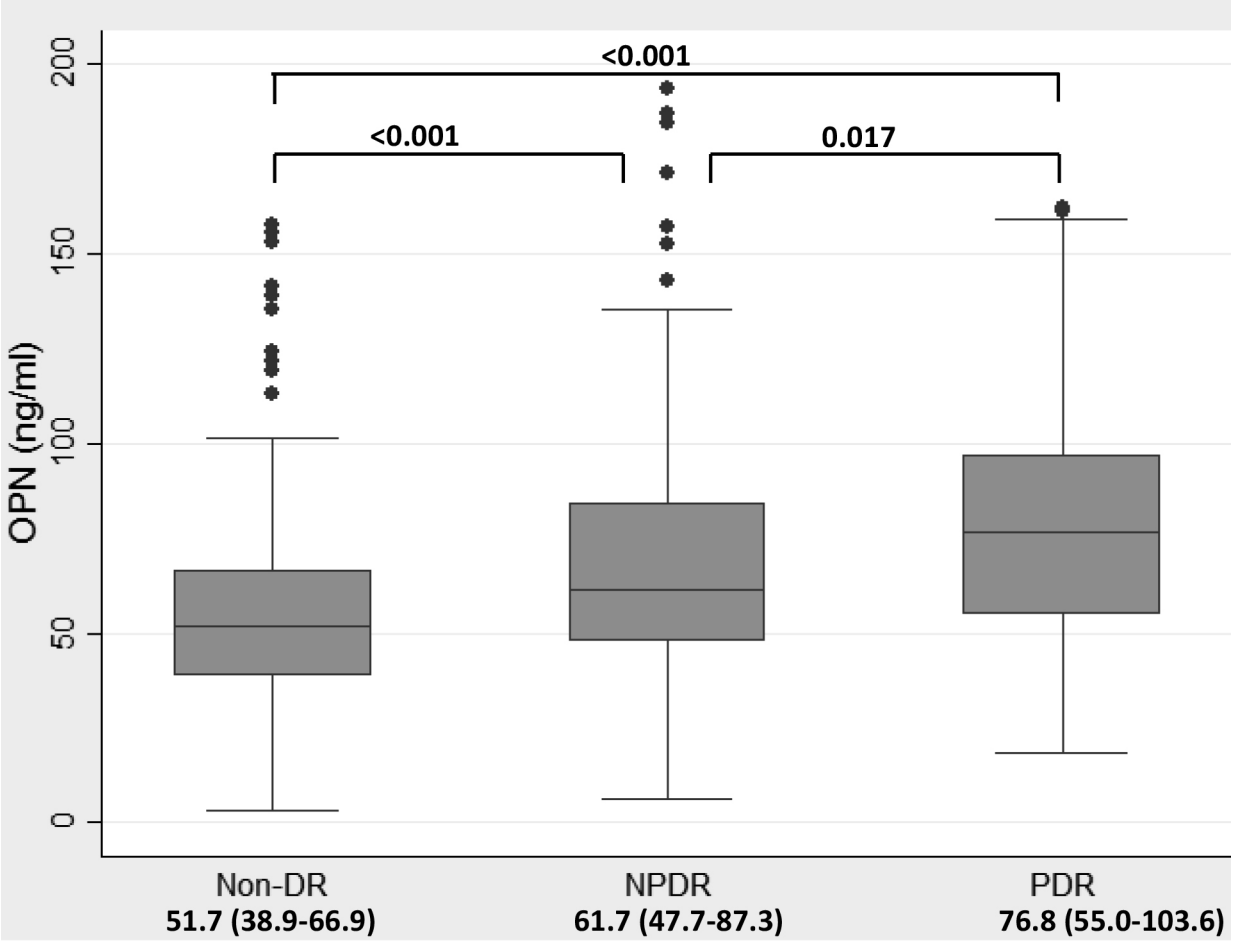

Figure 1. Box plot representing OPN levels in patients without DR, patients with NPDR, and patients with PDR. Compared with the median in patients without diabetic retinopathy (DR; 51.7 (38.9–66.9) ng/ml), the median of the OPN level is statistically significantly higher in patients with non-proliferative diabetic retinopathy (NPDR; 61.7 (47.7–87.3) ng/ml, p<0.001) and patients with proliferative diabetic retinopathy (PDR; 76.8 (55.00–103.6) ng/ml), p<0.001). The OPN level is also statistically significantly higher in patients with PDR than in patients with NPDR (p=0.017; Wilcoxon rank-sum test).

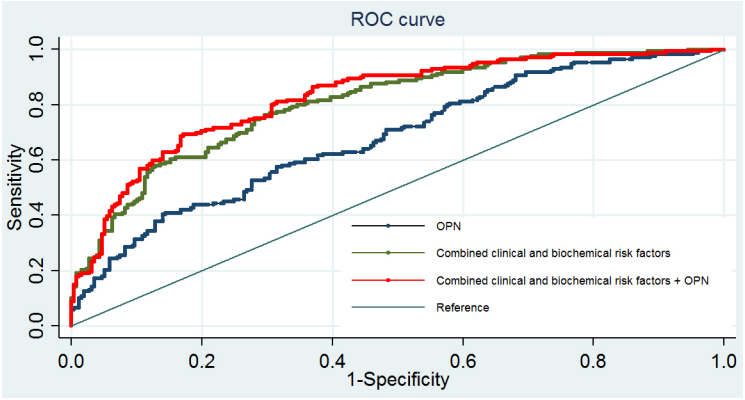

Figure 2. ROCs of OPN, variables (clinical and biochemical factors), and the combination of OPN plus variables to predict the presence of DR. Performance in predicting diabetic retinopathy (DR) was assessed by comparing the area under the curve (AUC) calculated before and after the addition of OPN levels to the model based on non-parametric approaches. We observed a statistically significant improvement in the AUC after OPN was added to the model (0.805 (95% confidence interval [CI], 0.763–0.846) versus 0.825, (95% CI, 0.785–0.865), p=0.011).

study, we observed a statistically significantly increased burden of other diabetic complications with DR severity, such as diabetic chronic kidney disease (35.8% versus 66.7% versus 85.7% for patients without DR versus patients with NPDR versus patients with PDR, respectively, p<0.001) and neuropathy (3.5% versus 23.3% versus 42.5% for patients without DR versus patients with NPDR versus patients with PDR, respectively, p<0.001), suggesting DR is a proxy for the total disease burden in patients with type 2 diabetes. To date, all studies that have measured OPN levels (vitreous and plasma) were cross-sectional, and therefore, it remains unclear whether changes in plasma OPN levels represent the cause or consequence of DR.

The process of angiogenesis is dependent on the dynamic balance between angiogenic stimulators (i.e., vascular endothelial growth factor, VEGF) and inhibitors (i.e., PEDF) [18]. Circulating levels of OPN may be an important factor in the modulation of angiogenic proliferation associated with advanced stages of DR [9]; however, the specific underlying mechanism remains unclear. Experimental studies have proposed that OPN may lead to angiogenesis by altering the levels of angiogenic stimulators [19,20] and/or inhibitors [21]. In the present study, we found higher levels of PEDF in plasma in patients with PDR (17.9 (15.4–23.2) ng/ml) than in patients with NPDR (16.3 (13.3–19.6) ng/ml, p=0.011) and patients without DR (15.1 (12.4–18.3) ng/ml, p<0.001). Our observations agree with previous findings of higher PEDF levels in patients with late proliferative stages of DR [9]. The investigators suggested that the antiangiogenic effect of PEDF may be limited to early stages of DR [22], and higher levels of PEDF likely occur as a secondary response to counteract the activity of the angiogenic stimulators, such as VEGF [9]. Moreover, there may be a positive regulatory feedback loop in late proliferative stages of DR where OPN-induced synthesis of PEDF may be the underlying mechanism for higher levels of PEDF associated with PDR. Studies on other angiogenic factors, such as VEGF, are needed to provide a more complete understanding of the relationships among OPN, angiogenesis, and DR.

Recent evidence suggests that chronic low-grade subclinical inflammation due to expression of numerous inflammatory markers may contribute to vascular lesions of DR [23]. Previous studies have also demonstrated increased expression of OPN in several chronic inflammatory diseases, such as atherosclerosis [23], suggesting inflammation as a link between OPN and DR. sRAGE is a multiligand receptor on vascular cells that plays a key role in inflammatory processes [24]. sRAGE, produced from RAGE either by proteolytic cleavage or alternative splicing, has a glycation end products (AGE)-binding property but lacks subsequent signaling properties [25]. The association of sRAGE and DR has been repeatedly reported in different populations [26,27]. Consistently, we found statistically significantly higher sRAGE levels in patients with PDR (953.4 (650.9–1467.7) pg/ml) than in patients with NPDR (766.3 (516.6–1140) pg/ml, p=0.01) and patients without DR (726.6 (525.9–1054.7) pg/ml, p<0.01), and the association of sRAGE with PDR remained after adjustment for the confounding factors (Table 2). It is believed that the increase in sRAGE is attributable to greater AGE and RAGE response through a positive feedback mechanism or more splicing from the full-length RAGE in response to retinal injury. We also found a correlation between sRAGE and OPN (rho=0.146, p=0.002), suggesting the increased sRAGE is in parallel with OPN under DR conditions. Given that sRAGE is correlated with OPN and remained an independent predictor for PDR, there is a possibility that sRAGE may be the underlying mechanism linking OPN with DR [28]. We need to measure additional inflammatory markers (i.e., TNF-α) to address the relationship of OPN, inflammation, and DR in patients with type 2 diabetes.

The precise role of OPN as a novel marker in the pathogenesis of DR remains unclear, and several mechanisms have been proposed in addition to angiogenesis and inflammation. OPN has been noted to be involved in retinal fibrosis, a process interplayed with angiogenesis and inflammation that usually occurs in late stages of DR [9]. In addition, animal studies showed improved insulin sensitivity by OPN neutralization independent of body composition or energy expenditure, implicating that the effect on insulin resistance or sensitivity could be an underlying mechanism for OPN association with diabetic complications [29,30]. In addition, several in vitro studies observed different functions of OPN fragments cleaved by matrix metalloproteinase (MMP) suggesting that the interaction between OPN and MMPs may contribute to the pathogenesis of DR [31,32].

The present findings have potentially important clinical implications. First, identification of biomarkers for early recognition of patients at risk for developing vascular complications of diabetes is of utmost importance. These study results suggest that OPN levels in plasma may be a potential novel biomarker to predict retinal vascular complications secondary to the diabetic disease process. Second, given that therapeutic agents (i.e., statins) can reduce the expression of OPN in vascular smooth muscle cells [33], there is a possibility that plasma OPN levels can be modulated by the therapeutic agents that may lead to alteration of the processes (i.e., angiogenic or inflammatory) involved in the pathogenesis of DR.

This is the first study to observe that plasma levels of OPN were statistically significantly associated with the presence and severity of DR after clinical and biochemical risk factors were taken into consideration. Moreover, OPN levels were measured using blood samples that are easily accessible in clinical and community settings unlike vitreous samples, and therefore, plasma OPN levels can be used as a potential screening tool for DR. We also recognize that this study has several limitations. First, all subjects were recruited from a restructured hospital and a community-based primary healthcare clinic. Whether these findings can be extended to the general population remains to be determined. Second, the cross-sectional design of this study precludes any causal inference between plasma OPN levels and DR. Finally, the fundus photographs were captured using a non-mydriatic camera, and therefore, it is possible that some cases of DR were not detected. In summary, these results demonstrate that higher levels of OPN in plasma are associated with the presence and severity of DR in patients with type 2 diabetes, suggesting OPN may be useful as a potential biomarker for DR.

APPENDIX 1. CHARACTERISTICS OF SELECTED AND UNSELECTED INDIVIDUALS WITH T2DM

To access the data, click or select the words "Appendix 1"

APPENDIX 2. UNIVARIATE ANALYSIS BETWEEN PRESENCE OF DR WITH CLINICAL AND BIOCHEMICAL VARIABLES

To access the data, click or select the words "Appendix 2"

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