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Osteopontin and clusterin levels in type 2 diabetes mellitus: differential association with peripheral autonomic nerve function

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Abstract

Osteopontin (OPN) and clusterin are secreted glycoproteins potentially associated with nerve function. Sudomotor dysfunction is associated with the development of foot ulcerations. The purpose of this study was to investigate the potential relationship of OPN and clusterin with sudomotor function (i.e., autonomic nerves that control sweating) in participants with type 2 diabetes mellitus (T2DM). Sudomotor function was assessed using SUDOSCAN® which measures electrochemical skin conductance (ESC) of the hands and feet. Demographics (e.g., age, gender, race, body mass index (BMI)), HbA1c, 25-hydroxyvitamin D, creatinine, OPN, and clusterin were also determined for the participants. Fifty individuals with T2DM (age = 59±11 years; 23/27 male/female; 13 African Americans) participated in this study. Lower ESC for the hands and feet were observed in African Americans versus Caucasians/Asians ($p < 0.05$). No significant ESC differences were observed for good [HbA1c <7%] versus poor [HbA1c ≥7%] glycemic control. With regard to gender, ESC values were lower for the hands for females ($p < 0.05$). In linear regression with ESC for the hands or feet as the dependent variable, increased OPN levels, but not clusterin, were independently associated with reduced sudomotor function while adjusting for age, gender, race, BMI, and glycemic control (ESC hands model $R^2 = 0.504$, $p < 0.001$; ESC feet model $R^2 = 0.534$, $p < 0.001$). The association between OPN and reduced sudomotor function found in our study warrants further investigation to delineate the underlying mechanisms and determine if OPN is neuroprotective, involved in the pathogenesis of sudomotor dysfunction, or simply a bystander.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Keywords

Osteopontin; Clusterin; Type 2 diabetes mellitus; Sudomotor function

Introduction

Osteopontin (OPN) and clusterin are systemic glycoproteins associated with many cellular functions. Schwann cell-derived OPN and clusterin are reported to have unique roles in peripheral motor and sensory axon regeneration in rodents [1]. The many roles that OPN performs include bone resorption, activation of immune cells, and inhibiting apoptosis [2]. With regard to nerve function, OPN was upregulated in denervated motor pathways in rodents [1]. Immunocytochemical analysis of sural nerve biopsies from humans with suspected or proven polyneuropathies has showed that Schwann cell expression of OPN is regulated by axon-derived signals [3]. Clusterin, a glycoprotein found in the central nervous system and plasma, has also been implicated in many physiological processes such as decreasing inflammation, protective role in cell survival, and lipid transport [4]. It is associated with neurodegenerative diseases (e.g., Alzheimer disease) with increased expression of clusterin potentially reflecting a neuroprotective response [5, 6]. In rodents, clusterin was upregulated in denervated sensory pathways [1].

Diabetic neuropathy is one of the most prevalent complications of diabetes [7]. The most studied forms of diabetic neuropathy are distal symmetric polyneuropathy and diabetic autonomic neuropathies. Distal symmetric polyneuropathy, via loss of small- and large-nerve fiber function, is a leading reason for recurrent injuries with an increased risk of falls and fractures. It is also a major cause for the development of foot ulceration [7]. In diabetic autonomic neuropathy, parasympathetic and/or sympathetic neurons may be affected leading to various clinical manifestations (e.g., orthostatic hypotension, hypoglycemia unawareness), including sudomotor dysfunction [7]. The examination of somatic and autonomic nerve fiber function has also been shown to be important predictors of cardiovascular risk [8].

Nerve fibers that innervate sweat glands are sudomotor, postganglionic, unmyelinated sympathetic C-fibers [9]. Sudorimetry is used to measure the function of sweat gland innervation. SUDOSCAN® is an instrument that evaluates sweat gland function via the measurement of electrochemical skin conductance (ESC) [10]. ESC is based on an electrochemical reaction between sweat chloride and stainless steel sensor plates in contact with palms of the hands and soles of the feet [10]. Assessing sudomotor function can be used to detect the presence of peripheral autonomic nerve fiber dysfunction [9]. Sudomotor dysfunction, an early neurophysiologic abnormality in diabetes, is associated with the development of foot ulceration [11]. Given the paucity of studies in humans exploring a likely association with sudomotor function and glycoproteins such as OPN and clusterin, the objective in this study was to determine the putative association among peripheral autonomic nerve fiber function, OPN, and clusterin in type 2 diabetes mellitus (T2DM).

Methods

Subjects

Fifty participants, who volunteered to participate in this study, were evaluated at the Diabetes and Metabolic Research Center, Christiana Care Health System, Newark, DE, USA. This study had approval of the Institutional Review Board of Christiana Care Corporation and each participant gave written informed consent before participating in the study. Individuals with T2DM were eligible for the study if they were 18 years old. Exclusion criteria included (a) history of known coronary artery disease or acute myocardial ischemia, (b) dose changes 2 months prior to enrollment for medications (e.g., antihypertensive, antidiabetes medications), (c) chronic kidney disease stage 3b, (d) any amputation other than involving the toes or fingers, and (e) any open wound on the soles of the feet or the palms of the hands.

Clinical measurements

The clinic stadiometer was used to measure weight and height. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). An oscillometric automatic recorder was used to measure blood pressure in the supine posture.

Sudomotor function testing

The SUDOSCAN® (Impeto Medical, Paris, France) device was used to measure sudomotor function of the hands and feet. Participants placed the palms of their hands and the soles of their feet on large area stainless steel sensor plates. The sensor plates were connected to a computer for recording and data management purposes. This non-invasive test required 3 min during which four combinations of 15 different low voltages ($< 4 \text{ V}$) of direct current were applied incrementally to the sensor plates. At voltages less than 10 V, the stratum corneum was electrically insulating and only sweat gland ducts were conductive. The instrument measured the ESC (i.e., ratio of the sweat chloride ion current measured over the constant power applied expressed in microSiemens (μS)) for the hands and feet.

Blood analytes

OPN and clusterin levels were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA). An electrochemiluminescence immunoassay was used to measure 25-hydroxyvitamin D levels. HbA1c was measured by high-performance liquid chromatography (HPLC) using a Tosoh G7 automated HPLC analyzer (Tosoh Bioscience, Inc., South San Francisco, CA, USA). Serum creatinine levels were determined by an enzymatic colorimetric assay.

Statistical analyses

Descriptive data are reported as mean $\pm$ SD for those variables normally distributed and median $\pm$ semi-interquartile ranges for those variables that are non-normally distributed. Bivariate comparisons of ESC for the hands and feet were made with the Mann-Whitney *U* test for males versus females, racial groups, and those with good versus poor glycemic control. Linear regression models with ESC for the hands and feet as the dependent variable

were used to test for independent associations of OPN and clusterin while adjusting for five covariates age, gender, race, BMI, and glycemic control. Normality was tested and, if violated, remedies were applied where appropriate.

Results

Table 1 provides the clinical characteristics of the participants. For participants with available clinical data ($n = 47$), approximately 30% of the individuals had peripheral sensory/motor neuropathy as determined via chart review of physician diagnosed signs and symptoms, 33% of the males had central autonomic neuropathy (e.g., erectile dysfunction), whereas no participants were noted to have peripheral autonomic dysfunction.

Table 2 reveals comparisons of measures of sudomotor function for males versus females, racial groups, and those in good glycemic control (i.e., HbA1c <7%) versus those poorly controlled. With regard to gender, ESC values for the hands and feet were lower for females than males. After controlling for race, however, there was no statistically significant difference between males and females for ESC of the feet ($p > 0.05$), but ESC of the hands remained significant ($p < 0.05$). In terms of race, Caucasians/Asians had higher ESC levels than African Americans for both the hands and feet (Table 2). When controlled for gender, ESC values for both the hands and feet remained significantly different between the racial groups ($p < 0.05$). With regard to glycemic control, sudomotor function for the hands and feet were slightly higher for those in good control but there were no statistically significant differences between the groups. Utilizing normative ESC values from the literature [8] that were defined by racial background, approximately 44% of the participants in this study had moderate to severe sudomotor dysfunction of the hands whereas approximately 20% had moderate to severe sudomotor dysfunction of the feet. It should be noted, however, that it was not possible to determine how closely the demographics of our study cohort matched the study cohorts of the published normative data.

Multiple linear regression (Table 3) was performed regressing ESC for the hands and ESC for the feet on OPN and clusterin while adjusting for age, gender, BMI, good [HbA1c <7%] versus poor [HbA1c $\geq$ 7%] glycemic control, and race (i.e., Caucasians/Asians versus African Americans). Both regression models were significant, $F(7, 42) = 6.09$, $p < 0.001$, model $R^2 = 0.504$, adjusted $R^2 = 0.421$ and $F(7, 42) = 6.86$, $p < 0.001$, model $R^2 = 0.534$, adjusted $R^2 = 0.456$, for the hands and feet, respectively. Additionally, results were consistent in both models where OPN was a significant determinant of sudomotor function, p values <0.05, whereas clusterin was not (Table 3). All interactions among variables were examined and only one, age by race, was significant for the ESC of the feet. The inclusion of this interaction term in the model did not change the effect of the results for the variables of interest (i.e., OPN and clusterin). Therefore, only the model without the interaction term was presented (Table 3).

Discussion

In the current study, we investigated whether OPN and clusterin were associated with sudomotor function while adjusting for demographic (e.g., age, race) and metabolic

parameters in individuals with T2DM. Interestingly, the results demonstrate that OPN, but not clusterin, is associated with reduced peripheral autonomic nerve function.

In peripheral autonomic neuropathy, secretion of sweat becomes dysfunctional and there is reduced sudomotor response with atrophy of sweat glands [11, 12]. Dysfunctional sweating contributes to a loss of humidification and cooling by evaporation resulting in dry skin [12, 13]. Patients consequently have reduced protective skin function which may lead to increased risk of injury and subsequent foot ulceration [12, 13]. In this study, SUDOSCAN® technology, based on electrochemical principles of reverse iontophoresis and chronoamperometry, was used to determine sweat gland function of the palms of the hands and soles of the feet [14]. The palms and soles were assessed because they have a high density of sweat glands [14] and neuropathy is frequently found in the extremities in persons with T2DM.

Nerve dysfunction in individuals with diabetes is complex, involving a cascade of pathogenic pathways triggered by hyperglycemia potentially leading to neuronal ischemia and death of cellular elements [15]. Metabolic insults and compromised blood flow to the nerve vascular supply have been implicated in pathogenic mechanisms with several factors potentially involved (e.g., formation of oxygen radicals, enhanced activity of the polyol pathway in neurons and Schwann cells, non-enzymatic glycosylation of neuronal structural proteins, neurotrophic disorder, pro-inflammatory changes) [12, 16–18]. In a different cohort of individuals with T2DM, we recently showed that increased OPN levels and the interaction with age had a significant association with reduced parasympathetic nerve function particularly in younger individuals, while adjusting for 25-hydroxyvitamin D insufficiency [19]. The results of the current investigation suggest an association between OPN and sudomotor dysfunction. The mechanism is, however, unclear as OPN, a widely expressed pleiotropic protein, functions in a number of physiological and pathologic conditions [20]. For example, OPN is involved in bone remodeling, wound healing, vascularization, response to ischemia, and inflammation [20, 21]. In rodents, OPN was upregulated in denervated motor pathways [1]. OPN is also involved in cell survival and may keep cells from undergoing apoptosis [22]. The association between increased OPN levels and reduced sudomotor function shown in the current study may be the result of increased synthesis in OPN as part of a compensatory mechanism (i.e., neuroprotective function) to limit nerve damage. It is also possible, however, that OPN could be part of a pathogenic process resulting in sudomotor dysfunction. For example, OPN is a pro-inflammatory cytokine secreted by many cell types and is also capable of modulating the expression of other inflammatory cytokines [23]. The chronic sustained release of OPN may prove toxic to neurons causing dysfunction and/or degeneration [23]. Given the cross-sectional design of the current study, a causal link between OPN and sudomotor function cannot be established.

We also examined a potential association between sudomotor dysfunction and circulating levels of clusterin in the current study. Clusterin, a secreted protein, is present in many physiologic fluids. Clusterin functions in the control of cell-matrix interactions, regulating apoptosis, transport of lipids, and regulation of complement [24, 25]. Clusterin is also a biosensor of oxidative injury and protects cells from the harmful effects of oxidative stress [26]. Increased clusterin levels have been found in several neurodegenerative diseases [24].

There was, however, no association of clusterin and sudomotor function found in this cohort of individuals with T2DM. Thus, even though OPN and clusterin have been shown in rodents to have unique roles in nerve function [1], only OPN was associated with sudomotor function in persons with T2DM in the current study. The functional heterogeneity of clusterin may play a role in this differential observation.

In performing our analysis in the current study, we took into consideration variables that might affect hands or feet conductance. For example, the effect of race on ESC in normal healthy subjects has been observed previously, with African Americans having values that are lower than Caucasians [27]. We observed similar results of race on ESC values in the current study in T2DM. One surprising finding in the current study was lower ESC values for the hands of females when compared with males. Our results are contrary to a study of healthy controls [27] and patients with distal symmetric polyneuropathy [28] where no difference between males and females was reported. An explanation for lower ESC values in the hands of T2DM females versus males found in the current study is not readily apparent, nor is the higher prevalence of moderate to severe sudomotor dysfunction in the hands versus feet. It should be noted that Parson et al. [29] recently showed in a study of individuals with T2DM that the expected distal to proximal gradient usually seen in diabetic peripheral neuropathy was not observed in their evaluation of small fiber function measured using contact heat-evoked potential stimulation. The observations with regard to ESC of the hands shown in our study warrant additional investigation, with larger studies being required to completely address these findings.

In addition to the cross-sectional nature of this study, there are a few other potential limitations that deserve mention as they should be considered while interpreting the data. In the regression models, we adjusted for glycemic control given that long-term hyperglycemia affects nerve function. Nonetheless, glycemic control was assessed by a single HbA1c and thus is only reflective of short-term glycemic control. It should also be noted that due to variant hemoglobins in two individuals, the degree of glycemia was estimated based on fructosamine levels. Repeat analysis of the regression models excluding these two individuals did not alter the main effects. Some participants may have been using medications that could have influenced sudomotor function, OPN, and/or clusterin. It should be noted, however, that there were no dose changes 2 months prior to enrollment for medications (e.g., antihypertensive and antidiabetes medications). The ESC measurements used in our study reflect the function of sweat glands and thus by inference, functional innervation [14]. Skin biopsies, which are invasive procedures, would have been needed to evaluate the association of OPN and a structural measure of sweat gland innervation [30].

In summary, we report a novel relationship between OPN and reduced peripheral autonomic function as assessed by sudomotor testing of the palms of the hands and the soles of the feet. To our knowledge, this is the first time that OPN, clusterin, and sudomotor function have been examined in individuals with T2DM. The discordant relationship of sudomotor dysfunction with OPN and clusterin found in the current study is intriguing. Considering the prevalence of nerve dysfunction in diabetes, the physiological and clinical significance of the data in the current study are important. With the exception of strict glycemic control [31], therapies to prevent or treat the progression of diabetic nerve dysfunction are few [32].

Thus, one potential clinical implication for the role of OPN is as a marker for the detection and/or treatment of nerve dysfunction. Accordingly, there is a crucial need to clarify in future studies whether OPN is neuroprotective, involved in the pathogenesis of sudomotor dysfunction, or merely a bystander. Specifically designed studies to delineate the underlying mechanisms involved and determine the putative role of OPN and sudomotor function are warranted.

Acknowledgments

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Table 1Descriptive statistics for general characteristics of the participants ($n = 50$)

Characteristics	
Age (years)	59 (11)
Duration of diabetes (years)	9 [6]
Male/female (n)	23/27
Caucasian/African American/Asian (n)	34/13/3
Body mass index (kg/m ²)	31.4 [3.7]
Systolic blood pressure (mmHg)	124 (13)
Diastolic blood pressure (mmHg)	75 (7)
Serum creatinine (μmol/L)	71 [13]
HbA1c (%)	7.0 [0.7]
25-Hydroxyvitamin D (nmol/L)	52 [25]
Osteopontin (ng/mL)	58 [10]
Clusterin (μg/mL)	210 (27)
Electrochemical skin conductance for the hands (μS)	63 [17]
Electrochemical skin conductance for the feet (μS)	78 [8]

Values presented as mean (SD) for variables that are normally distributed, median [semi-interquartile range] for variables non-normally distributed, or number of observations

Electrochemical skin conductance (ESC) of the hands and feet by demographics and glycemic control

Table 2

	Male (<i>n</i> = 23)	Female (<i>n</i> = 27)	<i>p</i> value	Caucasian/Asian (<i>n</i> = 37)	African American (<i>n</i> = 13)	<i>p</i> value	HbA1c <7% (<i>n</i> = 24)	HbA1c 7% (<i>n</i> = 26)	<i>p</i> value
ESC hands (μS)	74 ± 8	52 ± 21	<0.001*	68 ± 13	39 ± 18	<0.001***	64 ± 17	58 ± 19	0.977
ESC feet (μS)	83 ± 8	75 ± 10	0.026**	79 ± 6	65 ± 12	0.003***	79 ± 7	76 ± 11	0.648

Data are presented as median ± semi-interquartile range

* *p* value remained significant (*p* < 0.05) after controlling for race;

** *p* value was non-significant (*p* > 0.05) after controlling for race;

*** *p* values remained significant (*p* < 0.05) after controlling for gender

Table 3

Linear regression models for electrochemical skin conductance (ESC) of the hands and feet

Dependent variable	Independent variables	Regression coefficient	Standard error	<i>p</i> value
ESC hands	Osteopontin (ng/mL)	−0.221	0.096	0.026
	Clusterin (μg/mL)	−0.073	0.091	0.428
	Age (years)	0.159	0.237	0.506
	Gender	−12.835	5.254	0.019
	Race ^a	−13.968	5.727	0.019
	Body mass index (kg/m ²)	−0.719	0.404	0.082
	HbA1c category ^b	−6.664	4.634	0.158
ESC feet	Osteopontin (ng/mL)	−0.235	0.067	0.001
	Clusterin (μg/mL)	0.005	0.064	0.941
	Age (years)	0.744	0.167	<0.001
	Gender	−2.101	3.711	0.574
	Race ^a	−8.199	4.045	0.049
	Body mass index (kg/m ²)	−0.408	0.286	0.161
	HbA1c category ^b	−8.398	3.273	0.014

^aCaucasians/Asians versus African Americans^bHbA1c <7% (good glycemic control) versus HbA1c ≥7% (poor glycemic control)