Can J Diabetes 43 (2019) 336–344

Contents lists available at ScienceDirect

Canadian Journal of Diabetes

journal homepage: www.canadianjournalofdiabetes.com

Original Research

Associations of Serum S100B and S100P With the Presence and Classification of Diabetic Peripheral Neuropathy in Adults With Type 2 Diabetes: A Case-Cohort Study

Mohsen Afarideh MD, MPH [a]; Violet Zaker Esteghamati MD [a]; Morsaleh Ganji MD [a]; Behnam Heidari MD, MPH [a]; Sadaf Esteghamati MD [a]; Seyedsoroush Lavasani BSc [b]; Mona Ahmadi MD [c]; Abbas Tafakhori MD [c]; Manouchehr Nakhjavani MD [a]; Alireza Esteghamati MD [a,*]

[a] Endocrinology and Metabolism Research Center, Vali-Asr Hospital, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Virology, Iran University of Medical Sciences, Tehran, Iran
[c] Iranian Center for Neurological Research, Imam Khomeini Hospital Complex, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Key Messages

- Serum S100B and S100P were measured in patients with type 2 diabetes and peripheral neuropathy, and the levels were compared to those in patients with type 2 diabetes but without peripheral neuropathy.
- Serum S100P correlated with the presence and classification of diabetic peripheral neuropathy.
- S100B was not a sensitive indicator of diabetic peripheral neuropathy.
- S100P is a more significant indicator of diabetic peripheral neuropathy than S100B.

ARTICLE INFO

Article history:
Received 31 March 2018
Received in revised form
26 November 2018
Accepted 4 January 2019

Keywords:
classification
diabetic peripheral neuropathy
risk factor
S100B
S100P

ABSTRACT

Objectives: Novel biomarkers of diabetic peripheral neuropathy provide potentially useful information for early identification and treatment of diabetic neuropathy, ultimately serving to reduce the burden of disease. This study was designed to investigate the potential associations of serum S100B and S100P (calcium-modulated proteins) with the presence and classification of diabetic peripheral neuropathy in adults with type 2 diabetes.

Methods: In a case-cohort setting, the data of 44 participants diagnosed with diabetic peripheral neuropathy, 44 control participants with type 2 diabetes but free of peripheral neuropathy and 87 healthy control individuals were collected and analyzed.

Results: Serum S100P concentrations were elevated in participants with diabetic peripheral neuropathy compared with their controls with type 2 diabetes (median [IQR]: 2,235 pg/mL [1,497.5 to 2,680] vs. 1,200 pg/mL [975 to 1,350)], respectively; $p<0.001$). Conversely, serum S100B values were comparable in these 2 groups ($p=0.570$). Those with the typical diabetic peripheral neuropathy had significantly higher serum S100P levels compared to their counterparts with the atypical group of diabetic peripheral neuropathies ($p=0.048$). The independent significant association between serum S100P and diabetic peripheral neuropathy persisted into the multivariable adjusted logistic regression model (OR for S100P: 1.004 [95% CI 1.002 to 1.006]; $p<0.001$).

Conclusions: The present study's findings demonstrated that serum S100P is a more significant indicator of peripheral neuropathy in type 2 diabetes than is serum S100B. Prospective longitudinal studies are required to confirm the prognostic value of baseline serum S100P to predict incident peripheral neuropathy in people with diabetes.

* Address for correspondence Alireza Esteghamati, MD, Endocrinology and Metabolism Research Center, Vali-Asr Hospital, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran.
E-mail address: esteghamati@tums.ac.ir

1499-2671/
https://doi.org/10.1016/j.jcjd.2019.01.003

Mots clés:
classification
neuropathie diabétique périphérique
facteur de risque
S100B
S100P

RÉSUMÉ

Objectifs : Les nouveaux biomarqueurs de la neuropathie diabétique périphérique donnent des informations potentiellement utiles pour détecter et traiter de façon précoce la neuropathie diabétique, et permettent finalement de réduire le fardeau de la maladie. La présente étude a été conçue pour examiner les associations potentielles des concentrations sériques des protéines S100B et S100P (protéines modulées par le calcium) à la présence et à la classification de la neuropathie diabétique périphérique chez les adultes atteints du diabète de type 2.

Méthodes : Dans le cadre d'une étude cas-cohorte, nous avons collecté et analysé les données de 44 participants ayant un diagnostic de neuropathie diabétique périphérique, 44 participants témoins atteints du diabète de type 2, mais non de neuropathie périphérique, et 87 témoins en bonne santé.

Résultats : Les concentrations sériques de S100P étaient élevées chez les participants atteints de neuropathie diabétique périphérique comparativement aux témoins atteints du diabète de type 2 (intervalle interquartile [IIQ] médian: 2235 pg/mL [de 1497,5 à 2680] vs. 1200 pg/mL [de 975 à 1350)], respectivement; $p < 0{,}001$). Inversement, les valeurs sériques de S100B étaient comparables dans ces 2 groupes ($p = 0{,}570$). Les participants atteints de la neuropathie diabétique périphérique typique avaient des concentrations sériques de S100P significativement plus élevées que leurs homologues atteints de neuropathies diabétiques périphériques atypiques ($p = 0{,}048$). L'association significative indépendante entre les concentrations sériques de S100P et la neuropathie diabétique périphérique persistait dans le modèle ajusté de régression logistique multivariable (RIA pour les S100P: 1,004 [IC à 95 % 1,002 à 1,006]; $p < 0{,}001$).

Conclusions : Les résultats de la présente étude ont démontré que les concentrations sériques de S100P sont un indicateur plus significatif de neuropathie périphérique lors de diabète de type 2 que les concentrations sériques de S100B. Des études longitudinales prospectives sont nécessaires pour confirmer les valeurs pronostiques des concentrations sériques initiales de S100P dans la prédiction des nouveaux cas de neuropathie périphérique chez les personnes diabétiques.

Introduction

Diabetic peripheral neuropathy is 1 of the most common chronic microvascular complications of type 2 diabetes, rendering increased risks for active foot ulceration, tissue necrosis, Charcot joints and lower-extremity amputation because of causative nerve-function damage in the peripheral nervous system (1). Considering the very high burden of this diabetes-related microvascular complication, surrogate biomarkers of peripheral neuropathy are being sought actively in clinical research to refine early detection, halt progression and unearth novel targets for therapeutic intervention in patients with type 2 diabetes and peripheral neuropathy.

It has been suggested that a major cause of diabetic peripheral neuropathy is excessive oxidative stress due to long-term states of hyperglycemia at the outset and during the development of this condition (2). Various biomechanisms of oxidative stress occur because more oxidative agents are produced rather than the antioxidants secondary to the metabolites of chronic hyperglycemia, including the accumulating advanced glycation endproducts (2,3). In this process, the increasing formation of oxygen-free radicals interferes with the biologic function of neural cells via damage to the DNA, proteins and lipids, with the subsequent programming of neural cell death (3). The glial cells of the nervous system, which serve to maintain the equilibrium and protect neurons during the oxidative stress, are exposed to persistent cellular alterations and respond to these insults with upregulated astrocyte reactivity (4). During the astrocyte overactivity, the S100 calcium-binding protein B (S100B) is overproduced in response to the ongoing neural damage (5).

The S100B protein belongs to the superfamily of S100 cytoplasmic calcium-binding proteins that have both intra- and extracellular functions. This biomolecule plays a perceived dual protective-degenerative role in the nervous system (6). Indeed, the S100B protein participates in the survival of neurons as a predominantly trophic biofactor. On the other hand, overexpression of S100B contributes to mechanisms of neural cell apoptosis during the systemic neural insult (6). In addition, elevated serum levels of S100B may also reveal the overexpression of this protein secondary to the acute neural damage, e.g. trauma (7) and blood-brain barrier impairment (8,9).

Experimental studies have shown increasing local tissue levels of S100B and the glial fibrillary acidic protein to be markers of glial cell injury in the nervous systems of rats with diabetes. These reports indicated the association of astrocyte reactivity with oxidative stress in streptozotocin-treated rats with diabetes (10,11). Conversely, serum levels of the S100B protein were not associated with the progression of peripheral neuropathy in people with type 2 diabetes (6). A group of investigators demonstrated decreased serum levels of S100B in rats with diabetic ketoacidosis in comparison to healthy control rats and rats with diabetes but without diabetic ketoacidosis. The authors concluded that circulating S100B concentration is not a useful index for brain damage due to the occurrence of diabetic ketoacidosis (12).

Another member of the S100 superfamily is the S100 calcium-binding protein P (S100P), which is characterized by its various locations of gene polymorphisms compared to other members of this family (13). Raised circulating levels of the S100P protein have been associated with various cancers and their worst overall prognoses (14). The cell receptor for advanced glycation endproducts (RAGE) is activated by various ligands, such as the advanced glycation endproducts, S100s molecules and the amyloid proteins (15). This activation of RAGE by the S100s plays a key role in a number of important intracellular pathologic conditions (16). Importantly, the RAGE molecule is found on neural blood vessel walls (17), and studies have demonstrated a linkage between the expression of RAGE and peripheral neuropathy in animal models with diabetes (18,19). Therefore, altered expression of the S100P may be implicated in the pathogenesis of diabetic peripheral

neuropathy through activation of the advanced glycation endproducts-RAGE axis. To the best of our knowledge, no previous study has investigated the status of serum S100P in people with diabetic peripheral neuropathy.

Thus, we conducted the present study to: 1) comparatively assess serum levels of S100B and S100P proteins in various groups of people with diabetic peripheral neuropathy and their controls, both with and without diabetes; and 2) determine the potential association of S100B and S100P proteins with the presence and classification of diabetic peripheral neuropathy.

Methods

Study design, population and protocol

The population of the present case-cohort study was drawn from an ongoing biphasic cross-sectional and prospective observational study based in Tehran, the capital city of Iran (20–26). Details of original study design and population are outlined in the Supplementary Material. Between September 1 and December 31, 2016, a total of 257 consecutive people (142 people with type 2 diabetes and 115 individuals without diabetes) were formally requested to participate in the present study, of whom 205 individuals (109 with type 2 diabetes and 96 without diabetes) agreed to participate. Enrolled participants were excluded if they had histories of neurologic or neuromuscular disorders, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, subarachnoid hemorrhage, amyotrophic lateral sclerosis, cardiomyopathy or acute/chronic neuropathic syndromes associated with conditions other than diabetes (e.g. trigeminal neuralgia, postherpetic neuralgia, glossopharyngeal neuralgia, etc.), were taking medications (e.g. isoniazid) known to cause different forms of neuropathy or had family histories of neuropathies. Pregnancy, alcohol consumption, histories of surgery and/or trauma and previous or current diagnoses of active malignancies, chronic kidney disease, inflammatory or autoimmune disease, collagen vascular diseases, including systemic lupus erythematosus, hypothyroidism/hyperthyroidism, chronic obstructive pulmonary disease, hepatitis and/or other infections, and vitamin B_{12}/folate deficiency comprised other exclusion criteria of the present study.

Subsequently, data from a subsample of 175 eligible participants (44 with diabetic peripheral neuropathy, 44 with type 2 diabetes without peripheral neuropathy and 87 healthy controls; 90 men and 85 women; 24 to 75 years of age) were analyzed for this study. Considering the estimated prevalence of diabetic peripheral neuropathy in the original cohort (~30%), the study was designed to have 2 controls per case (i.e. 1 control with diabetes and 1 control without diabetes). Thus, the study power was estimated to be >99% with a type 1 error of 0.05 and a sample size of 175. Informed consent was obtained from all participants. The Institutional Review Board of the Tehran University of Medical Sciences approved the study's protocol.

Assessment of diabetic peripheral neuropathy

The entire subcohort of patients with type 2 diabetes was interviewed during every session about symptoms of sensory and motor neuropathy. The questionnaire was adapted according to diabetic neuropathy symptom scores, and patients were asked about experiencing newly developed pain, burning sensations, numbness and paresthesia of lower extremities and unsteadiness in walking during the past 2 weeks. Patients with diabetic neuropathy symptom scores above 1 were further assessed by our board-certified endocrinologists to suggest clinical diagnoses of diabetic peripheral neuropathy. Subsequently, a thorough diabetic peripheral neuropathy examination score adopted from Mythili (including a total of 8 tests, 2 assessing muscle strength, 1 studying tendon reflexes and 5 inspecting sensations) was performed to assess the status of diabetic peripheral neuropathy. Participants with diabetic neuropathy examination scores above 3 underwent electromyography and nerve conduction velocity (NCV) studies. The participants underwent conventional sensorimotor NCV studies performed by the same neurologist (AT), who was blinded to their diabetes status. The NCV study was performed at the ambient temperature (24°C) and at fixed distances on both the superior and inferior limbs (involving the median, ulnar, deep peroneal and tibial motor nerves and the median, ulnar and sural sensory nerves) by using the Medelec Synergy Electromyography device (Medelec Synergy, Oxford Instruments, Surrey, United Kingdom). The study of median, ulnar, deep peroneal and tibial nerves estimated the motor waves by generating distinct F waves. The sensory waves were calculated for median, ulnar and sural nerves. The H-reflex wave was also studied. Subsequently, the electromyography findings of proximal/distal latencies, amplitudes and NCVs of both motor and sensory nerves were determined. Thus, the diagnosis of diabetic peripheral neuropathy was confirmed based on abnormal values of distal sensory waves. The typical category of diabetic peripheral neuropathy is the most common variety in affected patients and is defined as the presence of a chronic, symmetrical, length-dependent sensorimotor polyneuropathy (i.e. diabetic sensorimotor polyneuropathy) (DSPN). Other types of peripheral neuropathies (i.e. non-DSPN) were classified as categories of atypical neuropathies (27).

Quantitative measurement of serum S100B and S100P levels

Serum S100B and S100P proteins (in dilution) were measured by ELISA kits according to the instructions of the manufacturer (Human Soluble Protein-100B [S100B] ELISA Kit, detection wavelength 450 nm, sample volume 50 to 100 μL, assay time 1 h to 5 h, #CSB-E08065h; Human S100 calcium binding protein P [S100P] ELISA Kit, detection wavelength 450 nm, sample volume 50 to 100 μL, assay time 1 h to 5 h, #CSB-E14080h; Cusabio Biotech, Wuhan, China). Duplicate evaluations were performed for each sample. The intra-assay and interassay conduction velocities for S100B and S100P ELISA kits were <8% and <10%, respectively. Serum levels of S100B and S100P proteins were calculated from a standard curve and expressed in picograms per milliliter (pg/mL), with normal reference ranges of 78 to 5000 pg/mL for serum S100B and S100P concentrations. The quality assessment of ELISA kits revealed the sensitivity of 19.5 pg/mL for serum S100B and S100P measurements.

Statistical analysis

The present study's quantitative variables are expressed as mean ± standard deviation (SD) or median (interquartile range) (IQR), based on the distribution of data (i.e. normal vs. abnormal distributions, respectively). Categorical variables are expressed as frequencies (%) or proportions and tested across groups by the chi-square statistic. The p for trend values of study variables across the groups of healthy individuals, controls with type 2 diabetes and participants with diabetic peripheral neuropathy were derived from the parametric linear regression models or the nonparametric Jonckheere-Terpstra test, as appropriate. The post hoc between-group comparisons of continuous variables with normal and abnormal distributions were carried out using the parametric Student t and nonparametric Mann-Whitney U tests, respectively. Bivariate correlations were tested employing the Spearman rank correlation coefficients (r). Serial stepwise models of logistic regression analysis were constructed to determine independent correlates of diabetic peripheral neuropathy among people

diagnosed with type 2 diabetes in crude and multivariate-adjusted models. Results of the logistic regression are presented as odds ratios (ORs) and 95% confidence intervals (95% CIs) and calculated per 1 unit increases in the serum values of S100B and S100P proteins and other continuous variables in all models. Baseline variables were considered in the multivariable logistic regression models if they were significantly different in those with diabetic peripheral neuropathy and their controls with type 2 diabetes but without the associated peripheral neuropathy or if they displayed a p value of less than 0.2 in the tests of between-group comparison. The optimal cutoff values of associated serum S100 proteins with their corresponding sensitivity/specificity pairs for the identification of diabetic peripheral neuropathy were determined based on plotting the potential receiver operating characteristic (ROC) curves. Only individuals with type 2 diabetes were selected for the ROC curve analysis to emphasize the power of serum S100s to demonstrate the progression of peripheral neuropathy in people with type 2 diabetes. The 2-sided statistical significance was defined at $p<0.05$ in all tests. All statistical tests were performed using the Statistical Package of Social Science software (SPSS) v. 18 (IBM, Chicago, Illinois, United States).

Results

Baseline characteristics of the population cohort

Data from 175 people, consisting of 3 groups of participants—with diabetic peripheral neuropathy (n=44), their controls with type 2 diabetes only (n=44) and healthy individuals (n=87)—were analyzed. The breakdown of those with diabetic peripheral neuropathy was made according to the following categories: DSPN, n=19, 43.2%); carpal tunnel syndrome (n=13, 29.5%), mononeuropathy (n=11, 25%) and radiculopathy (n=1, 2.3%). Of note, no patient in this study with diabetic peripheral neuropathy belonged to the category of mononeuritis multiplex (Table 1). Table 2 demonstrates baseline demographic, clinical and biochemical characteristics of the study's participants. Serum S100B and S100P values were not significantly different between men and women (median [IQR] of serum S100B: 207.5 pg/mL [158.25 to 282.75] vs. 221.5 pg/mL [170.25 to 276]; p=0.416; median [IQR] of serum S100P: 1465 pg/mL [1175 to 2237.5] vs. 1450 pg/mL [1152.5 to 2247.5]; p=0.821). Serum S100B and S100P concentrations displayed significant elevations over those of healthy controls and those of the controls with type 2 diabetes and the participants with diabetic peripheral neuropathy (p for trend value for S100B and S100P <0.001) (Table 2). The average serum levels of serum S100P were significantly higher in those with diabetic peripheral neuropathy than in the controls with type 2 diabetes but without coexisting peripheral neuropathy (median [IQR]: 2,235 pg/mL [1,497.5 to 2,680] vs. 1,200 pg/mL [975 to 1,350)], respectively; p<0.001) (Table 2). Conversely, serum S100B concentrations were comparable between participants with type 2 diabetes with or without coexisting peripheral neuropathy (p=0.570) (Table 2). In those diagnosed with diabetic peripheral neuropathy, the subset with typical diabetic peripheral neuropathy (i.e. DSPN) had significantly higher serum S100P values compared to the subset with the atypical group of diabetic peripheral neuropathies (median [IQR]: 2,450 pg/mL [1,840 to 3,040] vs. 1,990 pg/mL [1,390 to 2,340], respectively; p=0.048).

Correlates of serum S100B and S100P concentrations

Because the bivariate correlation coefficients of various study parameters with serum S100B and S100P values were not similar (data not shown here) across the 3 study groups of healthy control individuals, control people with type 2 diabetes and people with diabetic peripheral neuropathy, group-specific correlation coefficients were tested and are reported in the present study. The significant bivariate correlations were as follows: 1) S100B and high-density lipoprotein-cholesterol (HDL-C) ($r=-0.393$; p=0.008) in participants with diabetic peripheral neuropathy; 2) S100P and waist circumference ($r=-0.374$; p=0.012); S100P and SBP ($r=0.310$; p=0.040); and S100B and HDL-C ($r=0.348$; p=0.021) in controls with type 2 diabetes; and 3) S100P and total cholesterol ($r=0.236$; p=0.028); S100P and triglycerides ($r=0.328$; p=0.002); S100B and total cholesterol ($r=0.299$, p=0.005); S100B and HDL-C ($r=-0.225$; p=0.036); S100B and low-density lipoprotein-cholesterol (LDL-C) ($r=0.216$; p=0.044); and S100B and triglycerides ($r=0.497$; p<0.001) in healthy control individuals.

Serial logistic regression models of the association between S100P and diabetic peripheral neuropathy

Table 3 demonstrates results from the stepwise logistic regression models of the associations among serum levels of S100B and S100P and the presence of peripheral neuropathy in the subset of the study population with type 2 diabetes (n=88). In the crude logistic regression model, serum S100P values demonstrated a significant positive association with the presence of peripheral neuropathy in people affected by type 2 diabetes (OR for S100P in the crude model: 1.003 [95% CI 1.002 to 1.005]; p<0.001). Importantly, the independent significant association between increased serum S100P levels and the presence of diabetic peripheral neuropathy persisted into the multivariable adjusted logistic regression model with controlling for various demographic, clinical and biochemical characteristics of participants (OR for S100P in the final adjusted mode: 1.004 [95% CI 1.002 to 1.006]; p<0.001; OR for waist circumference in the final adjusted mode: 1.129 [95% CI: 1.032 to 1.236]; p=0.008).

Receiving operating characteristic curve for the identification of diabetic peripheral neuropathy according to serum S100P values

The receiver operating characteristic (ROC) curve for the ability of serum S100P values to diagnose peripheral neuropathy in the subset of the study population with type 2 diabetes is depicted in Figure 1. Consequently, the area under the ROC curve for serum S100P levels was calculated to be 0.896 ([95% CI 0.832 to 0.961]; standard error=0.033; p<0.001). In the sensitivity analysis, the clinical cutoff of serum S100P levels for the identification of peripheral neuropathy in people with type 2 diabetes was estimated at 1,305 pg/mL, with a sensitivity of 93.2% and a specificity of 70.5%. The positive predictive value and negative predictive value for this cutoff of serum S100P were determined at 75.9% and 91.2%, respectively. In the subgroup with type 2 diabetes, the fraction of the population with serum S100P levels above the calculated cutoff of 1,305 pg/mL had an approximate 32.5-fold increased risk of being concurrently affected by peripheral neuropathy (OR for serum S100P: 32.590 [95% CI 8.540 to 124.369]; p<0.001). Despite the multivariable adjustment by confounding factors in the finally adjusted model (Table 3), the study's paticipants maintained approximately 54.5-fold increased odds for the presence of diabetic peripheral neuropathy (OR for serum S100P: 54.514 [95% CI 7.857 to 378.249]; p<0.001).

Discussion

The present study demonstrated for the first time the significant positive correlation of serum S100P with the presence and classification of diabetic peripheral neuropathy. A sustained increase from the control group of healthy people to the controls with type 2 diabetes (i.e. type 2 diabetes without coexisting peripheral neuropathy) and participants with diabetic peripheral neuropathy suggests the possible involvement of serum S100P alterations in the

Table 1
Summary of electromyography and nerve conduction velocity results in the participants with diabetic peripheral neuropathy (n=44)

Patient	TA	GCN	FDI	VL	VM	BB	TB	RM	DL	PQ	TPS	Verdict and notes
1	R	R (medial head)	N	–	–	–	–	–	–	–	–	Axonal sensorimotor (sensory>motor) polyneuropathy with superimposed bilateral moderate carpal tunnel syndrome
2	R	R (medial head)	–	N	–	–	–	–	–	–	–	Chronic axonal sensorimotor polyneuropathy
3	N	N (medial head)	N	N	–	–	–	–	–	–	–	Mild distal sensory mononeuropathy with superimposed bilateral mild carpal tunnel syndrome
4	N	–	N	N	–	N	–	–	–	–	–	Bilateral moderate carpal tunnel syndrome
5	–	R (lateral head)	–	R	–	–	–	–	–	–	R	Subacute to chronic axonal sensorimotor polyneuropathy with superimposed bilateral moderate carpal tunnel syndrome
6	R	R (medial head)	R	–	–	–	–	–	–	–	–	Sensorimotor polyneuropathy with superimposed severed right-sided carpal tunnel syndrome
7	R	–	–	–	–	–	–	–	–	–	–	Chronic axonal sensorimotor polyneuropathy at lower extremity and mild left-sided carpal tunnel syndrome
8	N	–	–	–	–	–	–	–	–	–	–	Chronic predominantly sensorimotor axonal polyneuropathy with superimposed severe left-sided carpal tunnel syndrome and left-sided ulnar neuropathy
9	N	–	N	N	–	N	–	–	–	–	–	Mild distal sensory mononeuropathy
10	R	R (medial head)	–	N	–	N	–	N	–	–	–	Chronic L5–S1 radiculopathy without active process No evidence of neuropathy
11	R	–	–	–	–	–	–	–	–	–	–	Chronic axonal sensorimotor polyneuropathy
12	N	N (medial head)	N	–	–	–	–	–	N	–	–	Minimal carpal tunnel syndrome
13	N	–	N	N	–	–	–	–	–	–	–	Mild distal sensory mononeuropathy
14	N	–	N	N	–	–	–	–	–	–	–	Right moderate and left mild carpal tunnel syndrome
15	N	N (medial head)	N	–	–	N	–	–	–	–	–	Mild distal sensory mononeuropathy
16	N	N (medial head)	–	N	–	–	N	–	–	–	–	Bilateral mild carpal tunnel syndrome
17	R	–	R	–	–	–	–	–	–	–	–	Axonal sensorimotor polyneuropathy with active denervation
18	N	–	–	N	N	N	–	–	–	–	–	Sensory mononeuropathy
19	R	–	N	N	–	N	–	–	–	–	–	Chronic sensorimotor polyneuropathy
20	R	R (medial head)	–	N	–	–	–	–	–	–	–	Chronic mild sensorimotor polyneuropathy
21	N	N (medial head)	N	–	–	N	–	–	–	–	–	Distal sensory mononeuropathy with bilateral moderate carpal tunnel syndrome
22	N	N (medial head)	N	N	–	N	–	–	–	–	–	Right mild to moderate carpal tunnel syndrome
23	R	R (lateral head)	–	R	–	–	–	–	–	–	–	Axonal and demyelinating chronic sensorimotor polyneuropathy
24	R	R (medial head)	R	–	–	N	–	–	–	–	–	Subacute chronic axonal sensorimotor polyneuropathy
25	N	N (lateral head)	N	N	–	N	–	–	–	–	–	Right moderate and left mild carpal tunnel syndrome
26	N	–	N	N	–	N	–	–	–	–	–	Mild distal sensory mononeuropathy with right moderate and left moderate to severe carpal tunnel syndrome
27	R	R (medial head)	R	R	–	N	–	–	–	–	–	Subacute to chronic axonal sensorimotor polyneuropathy
28	N	–	N	N	–	N	–	–	–	–	–	Bilateral moderate carpal tunnel syndrome
29	–	–	–	–	–	–	–	–	–	–	–	Axonal right-sided median and ulnar neuropathy
30	N	N (medial head)	N	N	–	–	–	–	–	–	–	Bilateral moderate carpal tunnel syndrome
31	N	–	N	N	–	N	–	–	–	–	–	Bilateral mild carpal tunnel syndrome
32	N	N (medial head)	N	N	–	N	–	–	–	–	–	Sensory mononeuropathy with superimposed left moderate and right mild carpal tunnel syndrome
33	N	–	N	N	–	–	–	–	–	–	–	Mild distal sensory mononeuropathy
34	N	N (lateral head)	N	–	–	–	–	–	N	–	–	Mild left-sided carpal tunnel syndrome
35	N	–	–	N	–	N	–	–	–	–	–	Mild distal sensory mononeuropathy with superimposed mild right-sided carpal tunnel syndrome

(*continued on next page*)

Table 1 (*continued*)

Patient	TA	GCN	FDI	VL	VM	BB	TB	RM	DL	PQ	TPS	Verdict and notes
36	R	R (medial head)	R	R	–	N	–	–	–	–	–	Subacute to chronic sensorimotor polyneuropathy with superimposed right moderate carpal tunnel syndrome and right axonal ulnar neuropathy
37	R	R (medial head)	R	R	–	N	N	–	–	–	–	Bilateral chronic L3–L4–L5–S1 radiculopathy with superimposed bilateral moderate carpal tunnel syndrome and right-sided mild axonal ulnar neuropathy
38	R	–	N	N	–	N	–	–	–	–	–	Subacute to chronic axonal sensorimotor polyneuropathy
39	R	–	N	N	–	N	–	–	–	–	–	Chronic sensorimotor polyneuropathy
40	R	R (lateral head)	N	N	–	–	–	–	–	–	–	Axonal sensorimotor polyneuropathy in the lower limb
41	N	–	N	N	–	N	–	–	–	–	–	Moderate to severe bilateral carpal tunnel syndrome
42	N	–	N	N	–	N	–	–	–	N	–	Mild right-sided carpal tunnel syndrome
43	N	N (lateral head)	N	N	–	–	–	–	–	–	N	Moderate right-sided and mild left-sided carpal tunnel syndrome
44	R	R (medial head)	N	–	–	N	–	–	–	–	–	Chronic axonal sensorimotor polyneuropathy with superimposed moderate left-sided carpal tunnel syndrome

BB, biceps brachii; *DL*, deltoid; *FDI*, first digital interosseous; *GCN*, gastrocnemius; *N*, normal; *PQ*, pronator quadratus; *R*, reduced; *RM*, rhomboid major; *TA*, tibialis anterior; *TB*, triceps brachii; *TPS*, thoracic paraspinals; *VL*, vastus lateralis; *VM*, vastus medialis.

pathogenesis of peripheral neuropathy in type 2 diabetes. Conversely, serum concentrations of the S100B protein were comparable between type 2 diabetes controls and participants with diabetic peripheral neuropathy, although serum S100B levels were higher in controls with type 2 diabetes than in healthy individuals. In the multivariate logistic regression analysis, serum S100P concentrations were independently associated with the presence of diabetic peripheral neuropathy, harboring a 54.5-fold increased adjusted risk for the presence of peripheral neuropathy in the group of people with type 2 diabetes and with serum S100P values above the clinically determined cutoff of 1,305 pg/mL. Elevated average serum S100P levels were observed in those diagnosed with

Table 2
Baseline clinical, demographic and biochemical characteristics of the population cohort

Variable	Healthy controls (n=87)	Type 2 diabetes controls (n=44)	Diabetic peripheral neuropathy (n=44)	p for trend value	p diabetic controls vs. diabetic peripheral neuropathy
Gender (M/F)	42/45	21/23	27/17	0.196	0.199
Age (years)	38.81±6.99	52.50±8.67	56.57±8.54	<0.001	0.029
BMI (kg/m^2)	24.32 (23.36–24.80)	27.92 (25.75–32.50)	29.34 (27.43–33.19)	<0.001	0.179
Waist circumference (cm)	84.35±8.04	98.14±9.59	102.45±10.38	<0.001	0.046
Hip circumference (cm)	94.79±6.76	105.02±9.51	108.00±9.12	<0.001	0.138
SBP (mmHg)	107.84±11.02	127.16±15.60	135.79±17.55	<0.001	0.017
DBP (mmHg)	72.40±10.97	81.36±7.65	82.50±7.43	<0.001	0.482
Duration of diabetes (y)	–	8.50 (4.0–10.0)	10.0 (7.0–15.75)	N/A	0.009
FPG (mmol/L)	5.11 (4.77–5.44)	7.66 (7.17–9.80)	8.44 (7.12–11.42)	<0.001	0.193
2hPPG (mmol/L)	5.34±0.41	13.23±5.03	14.70±5.53	<0.001	0.196
A1C (%)	–	7.6 (6.8–8.34)	8.05 (7.12–9)	N/A	0.027
FPI (pmol/L)	–	74.66 (44.80–99.80)	66.32 (37.50–109.87)	N/A	0.608
HOMA-IR	–	3.36 (2.39–5.36)	3.45 (2.45–7.13)	N/A	0.796
Total cholesterol (mmol/L)	4.69±0.89	4.95±1.07	4.90±1.32	0.223	0.825
HDL-C (mmol/L)	1.35 (0.98–1.55)	1.22 (1.04–1.42)	1.04 (0.91–1.31)	0.004	0.030
LDL-C (mmol/L)	2.79±0.81	2.80±0.81	2.91±0.98	0.478	0.564
Triglycerides (mmol/L)	1.08 (0.85–1.50)	1.84 (1.39–2.37)	1.97 (1.35–2.57)	<0.001	0.619
ALT (μkat/L)	–	0.40 (0.30–0.61)	0.35 (0.28–0.46)	N/A	0.168
AST (μkat/L)	–	0.31 (0.27–0.42)	0.31 (0.25–0.40)	N/A	0.343
ALKP (μkat/L)	–	2.33 (1.77–3.01)	2.30 (1.59–3.19)	N/A	0.993
25(OH)D (nmol/L)	–	42.18 (25.51–54.29)	36.57 (22.71–49.92)	N/A	0.331
TSH (mIU/L)	–	1.82±0.87	1.80±0.64	N/A	0.948
UAE (mg/day)	–	12.5 (9.25–50)	29 (9.25–82.5)	N/A	0.176
Creatinine (μmol/L)	–	91.94±16.80	89.28±20.33	N/A	0.570
SUA (μmol/L)	220.09±24.98	352.75±96.37	367.02±89.23	0.130	0.463
Coexisting diabetic nephropathy (yes/no)	–	12/32	21/23	N/A	0.048
Coexisting diabetic retinopathy (yes/no)	–	6/38	18/26	N/A	0.004
Serum S100P (pg/mL)	660 (450–870)	1200 (975–1350)	2235 (1497.5–2680)	<0.001	<0.001
Serum S100B (pg/mL)	57.8 (48.7–90)	209 (168.25–291.75)	217.5 (158.5–261.25)	<0.001	0.570

ALKP, alkaline phosphatase; *ALT*, alanine aminotransferase; *A1C*, glycated hemoglobin; *AST*, aspartate aminotransferase; *BMI*, body mass index; *DBP*, diastolic blood pressure; *FPG*, fasting plasma glucose; *FPI*, fasting plasma insulin; *HDL-C*, high-density lipoprotein-cholesterol; *HOMA-IR*, homeostasis model assessment for insulin resistance; *kat*, katal, the catalytic activity that raises the rate of a chemical reaction by 1 mole per second; *LDL-C*, low-density lipoprotein-cholesterol; *N/A*, not applicable; *SBP*, systolic blood pressure; *SUA*, serum uric acid; *TSH*, thyroid stimulating hormone; *2hPPG*, 2-hour postprandial plasma glucose; *25(OH)D*, 25-hydroxyvitamin D; *UAE*, urinary albumin excretion.

Table 3
Stepwise models of logistic regression analysis demonstrating the multivariate associations of serum S100P and S100B with the presence of peripheral neuropathy in people with type 2 diabetes

Model	Predictive variables	Odds ratio	95% confidence interval	p value
Baseline (crude)	S100P	1.003	1.002–1.005	<0.001
	S100B	0.999	0.995–1.003	0.575
1 (adjusted by the variables of age, gender and waist circumference)	S100P	1.004	1.002–1.006	<0.001
	S100B	0.998	0.993–1.003	0.486
	Waist circumference	1.122	1.039–1.211	0.003
2 (additionally adjusted by the presence of coexisting nephropathy and retinopathy and duration of diabetes)	S100P	1.004	1.002–1.006	<0.001
	S100B	0.997	0.991–1.003	0.349
	Waist circumference	1.114	1.029–1.207	0.008
3 (additionally adjusted by the variables SBP and A1C)	S100P	1.004	1.002–1.006	<0.001
	S100B	0.997	0.991–1.004	0.418
	Waist circumference	1.116	1.027–1.214	0.010
4 (additionally adjusted by the variable HDL-C)	S100P	1.004	1.002–1.006	<0.001
	S100B	0.997	0.991–1.004	0.405
	Waist circumference	1.118	1.028–1.216	0.009
Multivariate adjusted (adjusted by the variables age, gender, waist circumference, presence of coexisting nephropathy and retinopathy, duration of diabetes, SBP, A1C, HDL-C, serum ALT, and UAE)	S100P	1.004	1.002–1.006	<0.001
	S100B	0.997	0.990–1.004	0.368
	Waist circumference	1.129	1.032–1.236	0.008

ALT, alanine aminotransferase; *A1C,* glycated hemoglobin; *HDL-C,* high-density lipoprotein-cholesterol; *SBP,* systolic blood pressure; *UAE,* urinary albumin excretion.

typical diabetic peripheral neuropathy (i.e. DSPN) compared to their counterparts affected with the atypical group of diabetic peripheral neuropathies (i.e. peripheral neuropathies other than DSPN).

S100 proteins are a glycoprotein superfamily with molecular weights ranging between 10 and 12 kilodaltons. They participate in fundamental surveillance of intracellular mechanisms by mediation of Ca^{2+}-dependent pathways, including the intracellular activation of enzymes through the protein phosphorylation and dephosphorylation and the hemostasis of calcium. The extracellular actions of S100 proteins have been attributed to the presence of RAGE reporters and are related to cell differentiation and apoptosis in the nervous system (28,29). Both the oxidative-induced apoptosis of neurocytes and the hyperglycemia-associated state of neuroinflammation, immune dysfunction and myelin impairment (e.g. via hyperactivity of the nuclear factor kappa B pathway) potentially result in the overactivity of astroglial cells and subsequently upregulated production of S100 proteins (2,30). Importantly, the multigenic family of S100 proteins is expressed in normal as well as in tumoral cell lines (14).

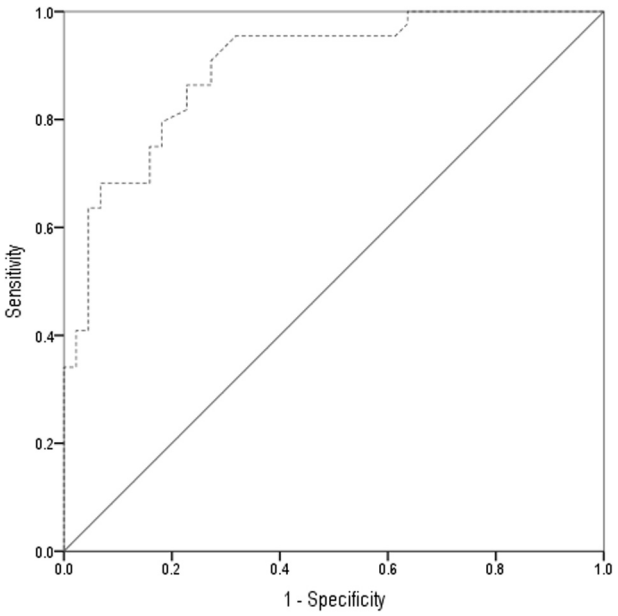

Figure 1. The receiver operating characteristic (ROC) curve of serum S100P values for the identification of peripheral neuropathy in people with type 2 diabetes.

The multiligand cell-surface RAGE has been associated with a diverse set of conditions, ranging from diabetes (29) to various types of cancers (31), inflammatory states (32) and neurologic disorders such as the Alzheimer disease (32,33). Activation of RAGE by advanced glycation endproducts in chronic hyperglycemia could lead to neural cell dysfunction and demise (34). In addition, the interaction between RAGE and nonadvanced glycation endproduct ligands such as the S100s may be a source of underlying neuropathy in diabetes (35) through their contribution to oxidative stress, activation of caspase-3 and changes induced in the DNA molecules (36). In a recent experimental study conducted in RAGE-knockout mice, milder stages of diabetic peripheral neuropathy were observed after 5 months of diabetes as opposed to the control RAGE +/+ group. Long-term observations have indicated a correlation between the increased expression of RAGE and the subsequent development of pathologic changes in the peripheral nervous system (19).

The cytoplasmic Ca^{2+} binding protein S100B is expressed in relatively abundant amounts by the glial cell astrocytes, Schwann cells and oligodendrocytes (37,38) of the central nervous system. In addition, the S100B protein is widely present in bodily tissues outside of the nervous system, such as skeletal muscle cells (39), adipocytes (40) and melanocytes (41). Overactivity of the astrocytes in response to persistent metabolic derangement is known to mediate the secretion of the S100B protein into the cerebrospinal fluid and, by crossing the blood-brain barrier, into the bloodstream (30). Alternatively, the compromised integrity of the blood-brain barrier is equally considered to cause elevation of serum S100B, even in the absence of apparent neural damage (9). Therefore, the S100B protein is usually not regarded as a glial-specific or brain-specific protein to signify early-stage neural impairment, particularly for pathologies of the peripheral nervous system.

In 2014, Celikbilek and associates were the first group to investigate the role of serum S100B alterations in diabetic peripheral neuropathy (6). Consistent with our findings, the authors reported no significant association between serum S100B levels and the presence of diabetic peripheral neuropathy in people with type 2 diabetes (6). They also observed markedly decreased serum S100B levels in people with type 2 diabetes compared to healthy controls. The authors attributed this observation to the

neuroprotective and beneficiary prosurvival effects of reduced serum S100B protein in its limited physiologic nanomolar concentrations as a salvage downregulation against the initial metabolic and glycemic insults in patients with new-onset diabetes, whereas the higher micromolar serum S100B concentrations are noted to introduce toxic neuroinflammatory and neurodegenerative effects through extensive neuronal and glial cell death and apoptosis during the later stages of chronic hyperglycemia (6). From this perspective, the significantly lower serum S100B concentrations in the study by Celikbilek, compared with the present study (10.889±2.787 pg/mL vs. 209.0 [168.25 to 291.75 pg/mL], respectively, albeit using different ELISA kits), is at least in part related to the use of different ELISA kits in our study and the study by Celikbilek and colleagues (6).

Previously, the S100P protein was investigated primarily for its role in the development as well as the progression of various cancers. Specifically, the protein S100P, which was originally localized from the human placenta (42), has been identified with key participations in the progression of hepatocellular carcinoma (43) as well as that of pancreas (44), colon (45), breast (46) and prostate (47) cancers. To the best of our knowledge, this is the first study to determine serum S100P alterations in diabetic peripheral neuropathy, reporting an independent association between raised serum S100P levels and the presence of this microvascular complication in diabetes as well as underlining the importance of serum S100P measurement in the classification of this condition.

We observed contrasting direct and inverse associations between serum S100B and HDL-C levels in controls with type 2 diabetes and participants with diabetic peripheral neuropathy, respectively. Because serum S100B levels were comparable between controls with type 2 diabetes and those with diabetic peripheral neuropathy, we postulate that the escalating serum release of S100B in patients with type 2 diabetes and early-stage neuroinflammation/neural impairment are, consequently, being largely suppressed by the compensatory upregulation of antioxidant HDL-C molecules (48) in response to the complicated metabolic and oxidative stress characterized by the progression of diabetic peripheral neuropathy. In light of these contrasting correlations, we suggest that future studies explore potential molecular links and common pathways mutually affecting serum concentrations of S100B and HDL-C. In addition, we noted an inverse significant correlation between serum S100P values and waist circumference in control participants with type 2 diabetes, and that was unexpected. In line with this, a recent study by Hou et al demonstrated that S100A4, as another member of the S100 calcium-binding protein family, is an inhibitory factor for obesity and attenuates the inflammatory reaction while activating protein kinase B signaling in adipose tissues. Based on this finding, the authors suggested that S100A4 is a potential candidate for the treatment of diet-induced obesity and its complications (49). Future studies are, therefore, required to further explore the possibility of any significant association in this regard and potentially to unearth viable biologic pathways linking S100P to obesity.

The present study had several important limitations. The cross-sectional nature of this study meant that the temporal order of association between the rise in serum S100B and the occurrence of diabetic peripheral neuropathy could not be established. In addition, concurrent assessment of other proposed correlates of neuropathy (e.g. examination of glial fibrillary acidic protein biomarkers of oxidative stress and inflammation) was not performed. Despite these limitations, the present study is strengthened by simultaneous investigation of S100B and S100P proteins, 2 distinct members of the S100 superfamily in a fairly large population of healthy individuals, type 2 diabetes controls and participants with diabetic peripheral neuropathy. Importantly, we comparatively determined serum S100B and S100P levels in the subgroups characterized by typical and atypical diabetic peripheral neuropathies.

Conclusions

In conclusion, the present study demonstrated independent associations of serum S100P with the presence and classification of diabetic peripheral neuropathy. In addition, those with the typical diabetic peripheral neuropathy had significantly elevated serum S100P levels compared to their counterparts with the atypical group of diabetic peripheral neuropathies. In comparison, serum S100B levels were not associated with the presence of peripheral neuropathy in people with type 2 diabetes, although serum S100B concentrations were elevated in controls with type 2 diabetes compared with healthy individuals. Therefore, serum S100P is suggested as a novel and sensitive indicator of peripheral neuropathy in people affected by type 2 diabetes (particularly with serum S100P values above the clinically determined cutoff of 1,305 pg/mL). Prospective longitudinal studies with larger sample sizes are required to establish the prognostic significance of baseline serum S100P levels as an independent risk factor for the development and progression of peripheral neuropathy in the group of people diagnosed with type 2 diabetes.

Author Disclosures

Conflicts of interest: None.

Author Contributions

MA (Mohsen Afarideh), VZE, MG, AT, MN and AE conceived the study, participated in its design, coordination, and acquisition of data. MA (Mohsen Afarideh) and MG wrote the manuscript and performed the statistical analysis. BH, SE, SL, and MA (Mona Ahmadi), participated in the review of literature. MA (Mohsen Afarideh), VZE, MG, AT, MN, and AE participated in the interpretation of the results and critical editing of the manuscript. All authors read and approved the final manuscript.

Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Diabetes* at www.canadianjournalofdiabetes.com.

References

1. Singleton JR, Smith AG. The diabetic neuropathies: Practical and rational therapy. Semin Neurol 2012;32:196–203.
2. Sandireddy R, Yerra VG, Areti A, Komirishetty P, Kumar A. Neuroinflammation and oxidative stress in diabetic neuropathy: Futuristic strategies based on these targets. Int J Endocrinol 2014;2014:674987.
3. Ullah A, Khan A, Khan I. Diabetes mellitus and oxidative stress—A concise review. Saudi Pharm J 2016;24:547–53.
4. Moghaddam HK, Baluchnejadmojarad T, Roghani M, et al. Berberine ameliorate oxidative stress and astrogliosis in the hippocampus of STZ-induced diabetic rats. Molec Neurobiol 2014;49:820–6.
5. Sofroniew MV, Vinters HV. Astrocytes: Biology and pathology. Acta Neuropatholog 2010;119:7–35.
6. Celikbilek A, Akyol L, Sabah S, et al. S100B as a glial cell marker in diabetic peripheral neuropathy. Neurosci Lett 2014;558:53–7.
7. Pelinka LE, Kroepfl A, Leixnering M, Buchinger W, Raabe A, Redl H. GFAP versus S100B in serum after traumatic brain injury: Relationship to brain damage and outcome. J Neurotrauma 2004;21:1553–61.
8. Donato R, Sorci G, Riuzzi F, et al. S100B's double life: Intracellular regulator and extracellular signal. Biochim Biophysic Acta 2009;1793:1008–22.
9. Kapural M, Krizanac-Bengez L, Barnett G, et al. Serum S-100β as a possible marker of blood–brain barrier disruption. Brain Res 2002;940:102–4.
10. Baydas G, Nedzvetskii VS, Tuzcu M, Yasar A, Kirichenko SV. Increase of glial fibrillary acidic protein and S-100B in hippocampus and cortex of diabetic rats: Effects of vitamin E. Eur J Pharmacol 2003;462:67–71.

11. Baydas G, Tuzcu M, Yasar A, Baydas B. Early changes in glial reactivity and lipid peroxidation in diabetic rat retina: Effects of melatonin. Acta Diabetolog 2004; 41:123–8.
12. Glaser N, Lo W, Tancredi D, Orgain M, Puvenna V, Janigro D. Levels of S100B in brain and blood of rats with diabetic ketoacidosis. Brain Res 2015;1624: 536–44.
13. Henry J, Toulza E, Hsu C-Y, et al. Update on the epidermal differentiation complex. Front Biosci 2012;17:1517–32.
14. Bresnick AR, Weber DJ, Zimmer DB. S100 proteins in cancer. Nature Rev Cancer 2015;15:96–109.
15. Nedić O, Rattan S, Grune T, Trougakos I. Molecular effects of advanced glycation end products on cell signalling pathways, ageing and pathophysiology. Free Rad Res 2013;47(Suppl 1):28–38.
16. Hsieh H-L, Schäfer BW, Weigle B, Heizmann CW. S100 protein translocation in response to extracellular S100 is mediated by receptor for advanced glycation endproducts in human endothelial cells. Biochem Biophys Res Commun 2004; 316:949–59.
17. Wada R, Yagihashi S. Role of advanced glycation end products and their receptors in development of diabetic neuropathy. Ann NY Acad Sci 2005;1043: 598–604.
18. Brussee V, Guo G, Dong Y, et al. Distal degenerative sensory neuropathy in a long-term type 2 diabetes rat model. Diabetes 2008;57:1664–73.
19. Toth C, Rong LL, Yang C, et al. Receptor for advanced glycation end products (RAGEs) and experimental diabetic neuropathy. Diabetes 2008; 57:2–17.
20. Hoseini SM, Kalantari A, Afarideh M, et al. Evaluation of plasma MMP-8, MMP-9 and TIMP-1 identifies candidate cardiometabolic risk marker in metabolic syndrome: Results from double-blinded nested case-control study. Metabolism 2015;64:527–38.
21. Afarideh M, Behdadnia A, Noshad S, et al. Association of peripheral 5-hydroxyindole-3-acetic acid, a serotonin derivative, with metabolic syndrome and low-grade inflammation. Endocr Pract 2015;21:711–8.
22. Afarideh M, Aryan Z, Ghajar A, et al. Complex association of serum alanine aminotransferase with the risk of future cardiovascular disease in type 2 diabetes. Atherosclerosis 2016;254:42–51.
23. Afarideh M, Ghajar A, Noshad S, Saadat M, Khajeh E, Esteghamati A. Serum 25-hydroxyvitamin D, non-alcoholic fatty liver disease and type 2 diabetes. Nutr, Metab Cardiovasc Dis 2017;27:93–5.
24. Afarideh M, Noshad S, Ghajar A, et al. Family history of diabetes and the risk of coronary heart disease in people with or without type 2 diabetes. Diabetes Metab 2017;43:180–3.
25. Esteghamati A, Momeni A, Abdollahi A, et al. Serum fibroblast growth factor 21 concentrations in type 2 diabetic retinopathy patients. Ann Endocrinol (Paris) 2016;77:586–92.
26. Esteghamati A, Khandan A, Momeni A, et al. Circulating levels of fibroblast growth factor 21 in early-stage diabetic kidney disease. Irish J Med Sci 2017; 186:785–94.
27. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: Update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 2010;33:2285–93.
28. Donato R. S100: A multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 2001;33:637–68.
29. Ramasamy R, Yan SF, Schmidt AM. Receptor for AGE (RAGE): signaling mechanisms in the pathogenesis of diabetes and its complications. Ann NY Acad Sci 2011;1243:88–102.
30. Baydas G, Reiter RJ, Yasar A, Tuzcu M, Akdemir I, Nedzvetskii VS. Melatonin reduces glial reactivity in the hippocampus, cortex, and cerebellum of streptozotocin-induced diabetic rats. Free Rad Biol Med 2003;35: 797–804.
31. Sparvero LJ, Asafu-Adjei D, Kang R, et al. RAGE (receptor for advanced glycation endproducts), RAGE ligands, and their role in cancer and inflammation. J Transl Med 2009;7:17.
32. Ray R, Juranek JK, Rai V. RAGE axis in neuroinflammation, neurodegeneration and its emerging role in the pathogenesis of amyotrophic lateral sclerosis. Neurosci Biobehav Rev 2016;62:48–55.
33. Cai Z, Liu N, Wang C, et al. Role of RAGE in Alzheimer's disease. Cell Molec Neurobiol 2016;36:483–95.
34. Piperi C, Goumenos A, Adamopoulos C, Papavassiliou AG. AGE/RAGE signalling regulation by miRNAs: Associations with diabetic complications and therapeutic potential. Int J Biochem Cell Biol 2015;60:197–201.
35. Manigrasso MB, Juranek J, Ramasamy R, Schmidt AM. Unlocking the biology of RAGE in diabetic microvascular complications. Trends Endocrinol Metab 2014; 25:15–22.
36. Vincent AM, Perrone L, Sullivan KA, et al. Receptor for advanced glycation end products activation injures primary sensory neurons via oxidative stress. Endocrinology 2007;148:548–58.
37. Hachem S, Aguirre A, Vives V, Marks A, Gallo V, Legraverend C. Spatial and temporal expression of S100B in cells of oligodendrocyte lineage. Glia 2005;51: 81–97.
38. Donato R, Cannon B, Sorci G, et al. Functions of S100 proteins. Curr Molec Med 2013;13:24–57.
39. Arcuri C, Giambanco I, Bianchi R, Donato R, Annexin V. S100A1 and S100B in developing and adult avian skeletal muscles. Neuroscience 2002;109: 371–88.
40. Gonçalves CA, Leite MC, Guerra MC. Adipocytes as an important source of serum S100B and possible roles of this protein in adipose tissue. Cardiovasc Psychiatr Neurol 2010;2010:790431.
41. Cheong KA, Noh M, Kim CH, Lee AY. S100B as a potential biomarker for the detection of cytotoxicity of melanocytes. Exper Dermatol 2014;23: 165–71.
42. Becker T, Gerke V, Kube E, Weber K. S100P, a novel Ca2+-binding protein from human placenta. Eur J Biochem 1992;207:541–7.
43. Yuan R-H, Chang K-T, Chen Y-L, et al. S100P expression is a novel prognostic factor in hepatocellular carcinoma and predicts survival in patients with high tumor stage or early recurrent tumors. PLoS One 2013;8:e65501.
44. Logsdon CD, Simeone DM, Binkley C, et al. Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. Cancer Res 2003;63:2649–57.
45. Birkenkamp-Demtroder K, Olesen SH, Sørensen FB, et al. Differential gene expression in colon cancer of the caecum versus the sigmoid and rectosigmoid. Gut 2005;54:374–84.
46. Peng C, Chen H, Wallwiener M, et al. Plasma S100P level as a novel prognostic marker of metastatic breast cancer. Breast Cancer Res Treatment 2016;157: 329–38.
47. Basu G, Kiefer J, Rojas A, et al. S100P promotes prostate cancer growth and survival. AACR 2007;67:5304.
48. Singh P, Mahadi F, Roy A, Sharma P. Reactive oxygen species, reactive nitrogen species and antioxidants in etiopathogenesis of diabetes mellitus type-2. Indian J Clin Biochem 2009;24:324–42.
49. Hou S, Jiao Y, Yuan Q, et al. S100A4 protects mice from high-fat diet-induced obesity and inflammation. Lab Invest 2018;98:1025–38.

Supplementary Material. Patients and Methods

Study design, population and protocol

The original population cohort comprised 2 subcohorts of patients with newly diagnosed type 2 diabetes (n=3,507) and the community-dwelling people without any type of diabetes (n=3,841), 18 to 75 years of age. Patients with type 1 diabetes, insulin-requiring patients with type 2 diabetes, people with gestational diabetes and people with diabetes secondary to malignant or metabolic conditions (e.g. diabetes in the settings of pancreatic cancer or pancreatitis, respectively) were excluded. For the present study, the group of patients with diabetic peripheral neuropathy and their control patients with type 2 diabetes were included from the diabetic subcohort; the healthy control individuals were participants in the subcohort without diabetes. Recruitment for this study began in 1995, and patients and data were collected until December 31, 2016 (n=7,348). Data from the patients registered prior to 2005 were recorded exclusively in a private diabetes clinic in Phase I of the study (the cross-sectional phase, 1994 to 2005) with the primary aim of providing sustained and high-quality care for referred individuals. The prospective program began systematic enrollment in 2005 and onward under the supervision of 4 collaborating health-surveillance centers located in the west, east, south and downtown regions of Tehran (Phase II). The primary goal of this second phase was to investigate the determinants and natural histories of the metabolic syndrome in a large representative population of the Tehran metropolitan area. People with type 2 diabetes began their treatment by lifestyle modification, metformin, glibenclamide/gliclazide and/or pioglitazone. The diagnosis of type 2 diabetes was established according to the American Diabetes Association criteria. Extensive details of the study's design and general characteristics of the patients have been reported elsewhere (1–5).

The study's population received regular periodic follow-up visits (scheduled at 3- to 6-month intervals) for the assessment of diabetes-control status as well as the annual determination of chronic microvascular complications of type 2 diabetes to investigate the pattern of common noncommunicable diseases and formulate a causal link between the wide range of studied health-related characteristics and the hard endpoint clinical outcomes. Individuals were visited at shorter notices if patients had complaints requiring attention at the clinic or the corresponding health-surveillance center. A conglomerate of demographic, clinical, biochemical and other traditional cardiometabolic-related risk factors, including the prescribed use of antihyperglycemic and lipid-lowering medications, and admission and hospital claim notes were assessed at baseline and during follow-up.

Definitions and terminology

The body mass indexes were calculated based on the Quetelet formula (kg/m^2). Homeostasis model assessment for insulin resistance was calculated as fasting plasma glucose (mg/dL) multiplied by fasting plasma insulin (U/L) divided by 405 (6). Diabetic retinopathy was defined primarily as advanced-stage retinopathy requiring laser treatment. In addition, those with scores greater than 20, as shown by fundus photography according to the Early Treatment Diabetic Retinopathy Study staging system or the Diabetic Retinopathy Disease Severity Scale score of 3, 4 or 5, as shown by dilated ophthalmoscopy, were considered positive for diabetic retinopathy in this study.

Our patients with type 2 diabetes and diabetic retinopathy, according to Volk or ocular lens (+90 or +78) indirect ophthalmoscopy using slit-lamp biomicroscopy (Topcon, Tokyo, Japan) and fluorescein angiography, ranged from nonproliferative diabetic retinopathy (with evidences of microaneurysms, hemorrhage and hard exudates) to proliferative diabetic retinopathy (newly formed blood vessels and/or growth of fibrous tissue into the vitreous cavity). Diabetic retinopathy was also considered positive in patients with moderate or severe maculopathy according to the Diabetic Macular Edema Disease (7) criteria. The Modification of Diet in Renal Disease formula was used to calculate the estimated glomerular filtration rate as follows: estimated glomerular filtration rate=$175 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times$ [0.742 if female] (8). Accordingly, diabetic nephropathy was defined by the presence of albuminuria (urinary albumin excretion≥30 mg/day confirmed by at least 2 samplings 3 to 6 months apart) or estimated glomerular filtration rate<60 mL/min/1.73 m^2 (9).

Clinical and biochemical measurements

Weights and heights were recorded with patients wearing light clothing and no shoes. The waist circumferences were measured by encircling the middle point between the iliac crest and the rib cage. Systolic blood pressure and diastolic blood pressure were measured twice after the patients rested for 5 to 10 min, and the mean values of these 2 measurements were recorded for the analysis. Venous blood samples were collected for biochemical analyses after an overnight 12-h fasting period. The glucose oxidase method (Pars Azmun, Karaj, Iran) was applied to measure the fasting plasma glucose and the 2-h postprandial plasma glucose. Fasting plasma insulin was measured by the radioimmunoassay technique (Immunotech, Prague, Czech Republic) with no crossreactivity for proinsulin or C-peptide with the antibody used. The intra-assay coefficients of variation for glucose and insulin were 2.1% and 4.3%, respectively. High-performance liquid chromatography (DS5 Pink kit; Drew, Marseille, France) was used to measure serum glycated hemoglobin. Enzymatic methods (Pars Azmun) were used to investigate the serum total cholesterol, triglycerides, low-density lipoprotein cholesterol and high density lipoprotein cholesterol. Patients were instructed to collect 24-h urine samples on 3 separate occasions within 3 to 6 months after the initial visit. The completeness of the collected samples was tested by measuring urinary creatinine excretion. A repeat measurement was requested if creatinine excretion levels were lower than 20 mg/kg per 24 h or 15 mg/kg per 24 h for men and women, respectively. Urinary albumin excretion was determined by calorimetric methods using commercial kits (ZiestChem Diagnostics, Tehran, Iran). Serum creatinine levels were determined by the Jaffe method (Pars Azmun). Serum liver function enzymes alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were performed using enzymatic photometry according to the International Federation of Clinical Chemistry and Laboratory Medicine (10) for serum alanine aminotransferase, aspartate aminotransferase; intra-assay coefficients of variation, 3.7% and 2.5%, respectively) and the Deutsche Gesellschaft für Klinische Chemie (11) for serum alkaline phosphatase; intra-assay coefficient of variation=1.5%) methods. The measurements were conducted using commercial Pars Azmun kits. Serum 25-hydroxyvitamin D was determined by using the enzyme-linked immunosorbent assay technique (Immundiagnostik, Bensheim, Germany).

Statistical analysis

The normality of data was tested against significance by using the numerical Shapiro-Wilk test followed by further clincial judgment based on the visualization of Q-Q plots and histograms.

One representative variable was entered in the multivariate models to reduce the multicollinearity effect in cases of 2 variables strongly related to each other. Collinearity diagnostics were run by exploring the tolerance and the variance inflation factor in a

criterion that is accompanied by examining the standard errors in each predictive model. In this definition, variance inflation factor >10/tolerance ≤0.1, condition index >30 and variance proportions >50% were carefully scrutinized for multicollinearity, allowing the appropriate covariates to enter the models with standard error values <2 for each independent variable. We included the best fitting model with the most robust Hosmer-Lemeshow statistic after separate forward, backward and stepwise selection approaches.

Supplementary References

1. Hoseini SM, Kalantari A, Afarideh M, et al. Evaluation of plasma MMP-8, MMP-9 and TIMP-1 identifies candidate cardiometabolic risk marker in metabolic syndrome: Results from a double-blinded nested case-control study. Metabolism 2015;64:527–38.
2. Afarideh M, Behdadnia A, Noshad S, et al. Association of peripheral 5-hydroxyindole-3-acetic acid, a serotonin derivative, with metabolic syndrome and low-grade inflammation. Endocr Prac 2015;21:711–8.
3. Afarideh M, Aryan Z, Ghajar A, et al. Complex association of serum alanine aminotransferase with the risk of future cardiovascular disease in type 2 diabetes. Atherosclerosis 2016;254:42–51.
4. Afarideh M, Ghajar A, Noshad S, Saadat M, Khajeh E, Esteghamati A. Serum 25-hydroxyvitamin D, non-alcoholic fatty liver disease and type 2 diabetes. Nutr Metab Cardiovasc Dis 2017;27:93–5.
5. Afarideh M, Noshad S, Ghajar A, et al. Family history of diabetes and the risk of coronary heart disease in people with or without type 2 diabetes. Diabetes Metab 2017;43:180–3.
6. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
7. Esteghamati A, Momeni A, Abdollahi A, et al. Serum fibroblast growth factor 21 concentrations in type 2 diabetic retinopathy patients. Ann Endocrinol (Paris) 2016;77:586–92.
8. Levey AS, Coresh J, Greene T, et al. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem 2007;53:766–72.
9. Esteghamati A, Khandan A, Momeni A, et al. Circulating levels of fibroblast growth factor 21 in early-stage diabetic kidney disease. Ir J Med Sci 2017;186:785–94.
10. Schumann G, Bonora R, Ceriotti F, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 4: Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. Clin Chem Lab Med 2002;40:718–24.
11. Thomas L, Müller M, Schumann G, et al. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum (Konsensus von DGKL und VDGH zu vorläufigen Referenzbereichen für Serumenzyme). Laborator Med 2005;29:301–8.