Study of serum S100P level and its relation to diabetic peripheral neuropathy in patients with type-2 diabetes

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Background
S100P, a binder of receptors for advanced-glycation end products, is an established biomarker of many types of cancer. However, data regarding its role in diabetes and diabetic peripheral neuropathy (DPN) are unclear.
Aim
The aim of this work was to study the relationship between serum S100P and DPN in patients with type-2 diabetes mellitus (T2DM).
Participants and methods
This cross-sectional study included a total of 90 subjects divided into three groups: 30 patients with T2DM complicated with peripheral neuropathy (group A), 30 patients with T2DM without peripheral neuropathy (group B), and 30 subjects as healthy-control group (group C). All patients with T2DM were assessed for peripheral neuropathy using Michigan neuropathy screening instruments and nerve-conduction study was done to diagnose subclinical neuropathy. Serum S100P was assessed by enzyme-linked immunosorbent assay technique.
Results
Mean serum S100P levels in group A and group B were significantly lower compared with group C ($P<0.001$ for both comparisons). However, there was no significant difference in mean serum S100P levels between groups A and B ($P=0.394$).
Conclusion
Serum S100P is significantly low in T2DM with no significant association with DPN.

Keywords:
biomarkers, diabetic peripheral neuropathy, s100p, type-2 diabetes mellitus

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Authors' contributions: All authors contributed to the study conception and design. Material preparation and data collection were performed by A.A. and data analysis was performed by T.AA, R.F., and M.R. Electrophysiological studies were done by R.E. The first draft of the paper was written by R.F. and all authors commented on previous versions of the paper. All authors read and approved the final paper.

Introduction

Diabetic neuropathies (DPNs) are the foremost prevalent chronic complications of diabetes affecting about 50% of patients. DPN is the most common type of neuropathy worldwide accounting for about 75% of the DPNs [1]. The reported prevalence of DPN ranges from 16% to as high as 87% [2]. DPN affects the distal nerves of the limbs, particularly those of the feet. It alters mainly the sensory function symmetrically causing abnormal feelings and progressive numbness that facilitates the formation of ulcers due to external trauma and/or abnormal distribution of the internal bone pressure [3]. Painful symptoms like burning, tingling, shooting, or stabbing are present in around one-third of DPN patients and approximately 20% of all diabetic patients [4]. These symptoms are generally worse at night and disturb sleep [5]. Along with painful symptoms during the day, this often leads to a decrease in individual's ability to perform daily activities [6].

The most critical complications of DPN include foot ulcers, Charcot foot abnormalities, injuries, and eventually, lower-extremity amputation, especially when concomitant peripheral vascular disease causes foot ischemia [7]. Amputation in people with diabetes is 10–20 times more common compared with that of nondiabetic people. Every 30 s, a lower limb or a part of a lower limb is lost to amputation somewhere within the world as a consequence of diabetes [8].

Therefore, there is an evident need for novel biomarkers of DPN not only to facilitate the early diagnosis and treatment, but also to clear the underlying pathophysiology of diabetic peripheral

neuropathy that might generate new therapeutic targets.

S100P, a 95-amino-acid protein, that is a member of the S100-family calcium-binding proteins, functions as an intracellular regulator of diverse cellular processes, including protein phosphorylation, cytoskeletal function, and protection from oxidative-cell damage [9–11].

S100P protein has received increasing attention as a result of accumulating evidence of its significant role during the development and progression of various cancers since its first association with human prostatic cancer [12].

Also, S100P can act in an autocrine manner through binding to the receptor for advanced glycation end products (RAGE) [13,14]. RAGE is the chief receptor through which AGE signaling, one of the most important pathophysiological mechanisms of diabetes and DPN, is mediated [15–17]. S100P, together with several other S100 proteins [18], acts as initial activator of the pathway via nuclear factor-κB/Rel complexes that translocate to the nucleus and induce the expression of several diverse target genes [19].

This activation of RAGE by the S100P is also a source of the underlying neuropathy in diabetes through their contribution to oxidative stress, activation of caspase-3, and changes induced within the DNA molecules [20]. So, distorted expression of the S100P might be implicated in the pathogenesis of DPN through activation of the advanced-glycation end products – RAGE alliance [20].

However, only one previous study examined the relation of serum S100P to DPN in subjects with T2DM. Therefore, the worth of S100P as a serum marker of DPN in patients with T2DM merits further studies. In this context, our aim was to assess the relation between DPN and serum S100P level in T2DM.

Materials and methods

Thirty patients (14 men and 16 women) aged between 36 years and 72 years with diabetic peripheral neuropathy (group A) and thirty patients (13 men and 17 women) aged between 35 years and 75 years with uncomplicated T2DM (group B) were enrolled in the current study among those attending the outpatient clinics of the Diabetes and Metabolism Department of Alexandria Main University Hospital. All patients had normal renal function (as assessed by urinary albumin–creatinine ratio and estimated glomerular filtration rate) and normal fundus examination. In addition, 30 apparently healthy subjects (14 men and 16 females) age-matched were included as a healthy-control group (group C). The study design was approved by the ethics committee of Alexandria University. The study was conducted according to the criteria set by the Declaration of Helsinki and each subject signed an informed consent before participating in the study.

Exclusion criteria

Patients with a history of nerve injury, patients with diabetic kidney disease or diabetic retinopathy, patients with autoimmune disease, thyroid disorder, malignancy, and alcohol consumption, infections such as hepatitis C virus, human immunodeficiency virus, or Epstein–Barr virus, and patients on medications that may cause peripheral neuropathy were excluded.

All participants underwent the following:

Clinical assessment

Full assessment of history was performed stressing on the duration of diabetes, neurological symptoms, presence of chronic illnesses, and detailed drug history. Body mass index was calculated using the Quetelet formula (weight in kg divided by height in m^2).

Clinical assessment of DPN

All patients with T2DM were assessed for peripheral neuropathy using Michigan neuropathy screening instruments that is composed of two parts: history of neuropathic symptoms and physical examination to assess the appearance and sensation of feet. Physical assessment includes the appearance of feet (presence of deformities, dry skin, callus, infection, and fissure), presence of ulceration, ankle reflexes, vibration perception at great toes, and assessment by monofilament. Abnormality in each item gets grades 0.5–1 and a minimum of more than 2 abnormal items are needed to achieve the score of neuropathy [21].

Electrophysiological assessment of DPN

All patients with T2DM underwent nerve-conduction study to incorporate patients with clinical and subclinical peripheral neuropathy and assess its type and severity. The nerve-conduction study was performed in the Department of Physical Medicine, Rheumatology and Rehabilitation in Alexandria Main

University Hospital using Nicolet Viking Quest version 11 USA electromyography machine. During the nerve-conduction study, the skin was kept warm around 32–34°C by using hot packs or infra-red lamp whenever needed. The nerve-conduction study was performed for tibial and peroneal motor nerves with their late responses (F-wave) as well as sural sensory nerve.

Biochemical analysis and quantitative measurement of serum S100P

Blood samples were taken at identical time of the day for all subjects. Blood samples were centrifuged within 20 min of collection at 37°C. Serum was separated from cells immediately after centrifugation and stored at −20°C until analyzed. The estimated glomerular filtration rate was measured using The Chronic Kidney Disease Epidemiology Collaboration equation.

Serum S100P was measured by the use of a commercially available double-antibody sandwich enzyme-linked immunosorbent assay kit (Shanghai Sunredbio (SRB) Technology Co. Ltd., Shanghai, China) in keeping with the manufacturer's instructions. S100P measurements are reported in picogram per milliliter (pg/ml).

Statistical analysis

Data are presented as mean±standard deviation (SD) or median (interquartile range). The Student's *t*-test was used to compare parameters between the two diabetic groups. To compare between more than two studied groups, Kruskal–Wallis test was used for abnormally distributed quantitative variables, analysis of variance test was used for normally distributed quantitative variables, and post hoc (Dunn's multiple-comparison test) for pairwise comparisons. Mann–Whitney *U* test was used for abnormally distributed quantitative variables, to compare between two studied groups. χ^2 test was used to compare categorical variables between groups. Spearman correlation analysis (nonnormal distribution) was used to assess the correlations between serum S100P and other continuous parameters. All statistical analyses were done using the SPSS software (version 20.0, IBM Corporation, Armonk, New York, USA).

The usefulness of serum S100P as a marker of peripheral neuropathy in T2DM and a marker uncomplicated T2DM was analyzed by the use of a receiver-operating characteristic (ROC) curve. A *P* value less than 0.05 was considered significant.

Results

Baseline demographic and clinical characteristics of the study population

There was no significant difference in gender, age, and body mass indexbetween the three studied groups. Also, there was no significant difference in HbA1c% between the two diabetic groups (group A and group B), but there was a statistically significant difference between them as regards the duration of diabetes 9.37 ±5.58 versus 5.39±6.14 years, *P*=0.004 (Table 1).

Serum S100P levels

There was a significant decrease in mean serum S100P in T2DM patients with DPN compared with the healthy-control group. Similarly, there was a significant decrease in mean serum S100P in T2DM patients without DPN compared with the healthy-control group. On the other hand, there was no significant difference in mean serum S100P levels between diabetic patients with and without DPN (Table 2).

Cutoff value of serum S100P that can identify DPN

An ROC curve analysis was performed to evaluate the usefulness of S100P as a marker for DPN. It showed an area under the curve of 0.8 ($P<0.001$) for T2DM with DPN. A concentration of less than or equal to 1800 pg/ml showed a specificity of 70% and a sensitivity of 80%

Table 1 Demographic, clinical, and laboratory findings of the studied subjects

	Group-A T2DM patients with DPN	Group-B T2DM patients without DPN	Group-C healthy controls	*P* value
N	30	30	30	
Men/women	14/16	13/17	14/16	0.956
Age (years), mean±SD	53.93±8.59	50.10±8.40	53.23±7.52	0.161
Duration of diabetes (years), mean ±SD	9.37±5.58	5.39±6.14	–	0.004*
BMI (kg/m^2), mean±SD	30.04±5.09	29.23±6.0	28.68±3.77	0.576
MNSI/10, mean±SD	5.23±1.68	1.67±1.49	–	<0.001*
HbA1c (%), mean±SD	8.86±1.83	8.10±1.17	–	0.145

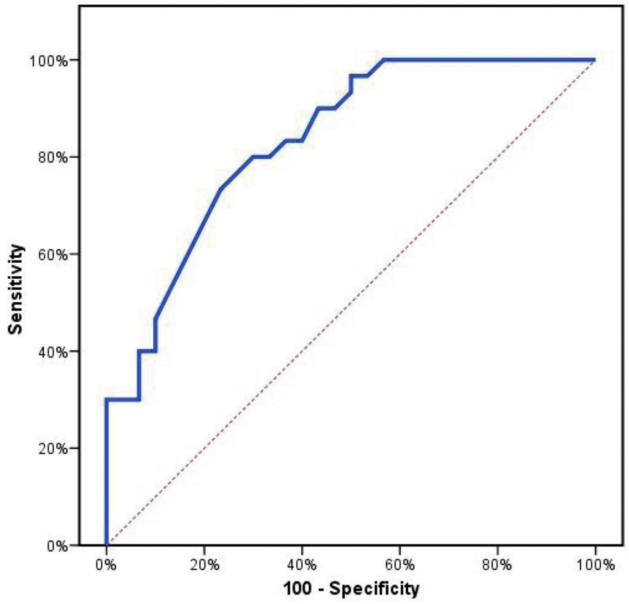
BMI, body mass index; MNSI, Michigan neuropathy screening instrument; SD, standard deviation. *Statistically significant at $P\leq0.05$.

Table 2 Comparison between the three studied groups according to serum S100P level

S100P (pg/ml)	Group A (*n*=30)	Group B (*n*=30)	Group C (*n*=30)	*P*
Mean±SD	1625.3±351.1	1828.7±1103.4	3310.0±2405.46	<0.001*
Median (IQR)	1700.0 (1420.0–1800.0)	1515.0 (1170.0–1900.0)	2100.0 (1800.0–4150.0)	
Significance between groups		P_1=0.394, P_2<0.001*, P_3<0.001*		

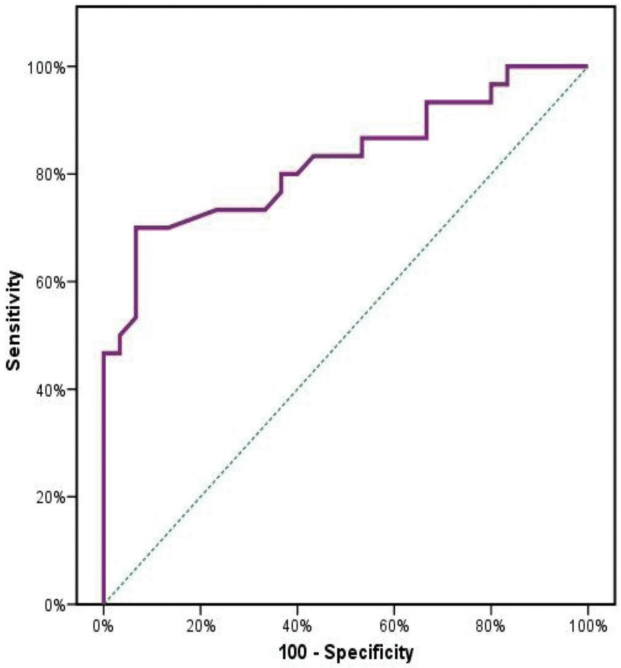
P: *P* value for comparing between the three studied groups. P_1: *P* value for comparing between group A and group B. P_2: *P* value for comparing between group A and group C. P_3: *P* value for comparing between group B and group C. *Statistically significant at $P \leq 0.05$.

Figure 1



ROC curve for S100P concentration (pg/ml) to diagnose DPN.

Figure 2



	S100P (pg/ml)
AUC	0.826*
P	<0.001*
95% C.I	0.718-0.933

ROC curve for S100P concentration to diagnose T2DM. AUC=area under the curve, CI=confidence interval. *Statistically significant at $P \leq 0.05$.

to identify T2DM with DPN from healthy control. The diagnostic performance of S100P revealed a sensitive rather than a specific marker (Fig. 1).

Cutoff value of serum S100P that can identify T2DM

ROC-curve analysis was performed to evaluate the usefulness of S100P as a marker for uncomplicated T2DM. It showed an area under the curve of 0.8 (P<0.001) for T2DM without DPN. A concentration of less than or equal to 1630 pg/ml showed a specificity of 93.3% and a sensitivity of 70% to identify T2DM without DPN from healthy control. The diagnostic performance of S100P revealed a specific rather than a sensitive marker (Fig. 2).

Discussion

Our study showed that lower S100P levels are associated with T2DM as compared with those of the healthy subjects. On the other hand, there was no significant association between S100P levels and diabetic peripheral neuropathy. The results raise the possibility for the usefulness of S100P as a serum marker for T2DM.

S100P is one of the few S100 proteins that act extracellularly through binding to RAGE [13]. S100P protein has gained increasing attention due to accumulating evidence of its significant role during the development and progression of different cancers since its first association with human prostate cancer [12]. To our knowledge, there is only one study conducted by Afarideh M, which investigated the level of serum S100P in patients with diabetic peripheral neuropathy. In disagreement with our results, they showed statistically significant sustained increase from the control group of healthy people to the controls with type-2 diabetes without peripheral neuropathy and participants with diabetic peripheral neuropathy [22].

We have to address some differences in Afarideh *et al.* study compared with ours. In Afarideh M, out of 44 DPN patients, 21 patients had coexisting diabetic nephropathy and 18 patients had coexisting diabetic retinopathy, while in our study, both diabetic nephropathy and retinopathy were excluded during sample selection. In addition, different populations and different S100P enzyme-linked immunosorbent assay kits were used, thus suggesting the different serum S100P levels [22].

The results of studies about S100B, another member of S100 proteins that binds to RAGE in the same manner as S100P, go hand in hand with our results [23,24]. There was a significant decrease in serum S100B levels in patients with T2DM compared with control participants, but no association between serum S100B levels and the presence of diabetic peripheral neuropathy or other microvascular complications was observed [23]. Also, the amount of S100B was markedly depleted from the common peroneal nerve of the diabetics when compared with nondiabetics [24].

Oxidative stress has been considered as a major hallmark for the pathogenesis and development of T2DM and DPN [25]. As a result of prolonged hyperglycemia, oxidative superoxides occur at abnormally high levels due to increased glycolysis and lipolysis [25]. AGE, polyol, protein-kinase C, and hexosamine pathways directly alter the redox capacity of the cell through formation of reactive oxygen species, leading to severe cellular oxidative stress and dysfunction [26]. High levels of reactive oxygen species and H_2O_2 result in peroxidation and nitrosylation of proteins, lipids, and nucleic acids [25]. Antioxidant-response element (ARE) is a *cis*-regulatory element or enhancer sequence, which is found in the promoter region of several genes encoding detoxification enzymes and cytoprotective proteins [27]. AREs are well-established as significant regulators of redox homeostasis and activators of cytoprotection during oxidative stress [28]. A putative ARE located at −1131 to −1122 from the transcriptional start site of S100P was found by DNA-sequence analysis [29].

The association between S100P and oxidative stress was demonstrated by a previous study and can be explained by that overproduction of reactive oxygen species can cause mutation of ARE on S100P gene that leads to suppression of S100P expression [30]. This could partly explain the downexpression of S100P in T2DM patients.

Conclusion

In summary, our results suggest that serum S100P is significantly low in T2DM with no significant association with DPN. Our observation that T2DM patients are presented with decreased levels of S100P suggests that S100P may be involved in the pathophysiological process of T2DM and can be used as a specific marker for T2DM.

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Conflicts of interest

There are no conflicts of interest.

References

1 Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, *et al.* Diabetic neuropathy: a position statement by the American Diabetes Association. Diabetes Care 2017; 40:136–154.

2 Boulton AJ, Armstrong DG, Albert SF, Frykberg RG, Hellman R, Kirkman MS, *et al.* Comprehensive foot examination and risk assessment. Diabetes Care 2008; 31:1679–1685.

3 International Diabetes Federation. IDF Diabetes Atlas. 9th ed. Brussels: International Diabetes Federation; 2019.

4 Tesfaye S. Recent advances in the management of diabetic symmetrical polyneuropathy. J Diabetes Invest 2010; 2:33–42.

5 Quattrini C, Tesfaye S. Understanding the impact of painful diabetic neuropathy. Diabetes Metab Res Rev 2003; 19: S2–S8.

6 van Deursen RW, Sanchez MM, Ulbrecht JS, Cavanagh PR. The role of muscle spindles in ankle movement perception in human subjects with diabetic neuropathy. Exp Brain Res 1998; 120:1–8.

7 Morrison S, Colberg SR, Mariano M, Parson HK, Vinik AI. Balance training reduces falls risk in older individuals with type 2 diabetes. Diabetes Care 2010; 33:748–750.

8 Moxey PW, Gogalniceanu P, Hinchliffe RJ, Loftus IM, Jones KJ, Thompson MM, *et al.* Lower extremity amputations – a review of global variability in incidence. Diabet Med 2011; 28:1144–1153.

9 McNutt NS. The S100 family of multipurpose calcium-binding proteins. J Cutan Pathol 1998; 25:521–529.

10 Schafer BW, Heizmann CW. The S100 family of EFhand calcium-binding proteins: functions and pathology. Trends Biochem Sci 1996; 21:134–140.

11 Zimmer DB, Cornwall EH, Landar A, Song W. The S100 protein family: history, function, and expression. Brain Res Bull 1995; 37:417–429.

12 Averboukh L, Liang P, Kantoff PW, Pardee AB. Regulation of S100P expression by androgen. Prostate 1996; 29:350–355.

13 Arumugam T, Simeone DM, Schmidt AM, Logsdon CD. S100P stimulates cell proliferation and survival via receptor for activated glycation end products (RAGE). J Biol Chem 2004; 279:5059–5065.

14 Fuentes MK, Nigavekar SS, Arumugam T, Logsdon CD, Schmidt AM, Park JC, *et al.* RAGE activation by S100P in colon cancer stimulates growth, migration, and cell signalling pathways. Dis Colon Rectum 2007; 50:1230–1240.

15 Brownlee M. Advanced protein glycosylation in diabetes and aging. Annu Rev 1995; 46:223–234.

16 Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia 2001; 44:129–146.

17 Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. Am J Physiol Endocrinol Metab 2001; 280: E685–E694.

18 Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, *et al.* RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. Cell 1999; 97:889–901.

19 Pahl HL. Activators and target genes of Rel/NFkappaB transcription factors. Oncogene 1999; 18:6853–6866.

20 Vincent AM, Perrone L, Sullivan KA, Backus C, Sastry AM, Lastoskie C, *et al.* Receptor for advanced glycation end products activation injures primary sensory neurons via oxidative stress. Endocrinology 2007; 148:548–558.

21 Herman WH, Pop-Busui R, Braffett BH, Martin CL, Cleary PA, Albers JW. Use of the Michigan Neuropathy Screening Instrument as a measure of distal symmetrical peripheral neuropathy in type 1 diabetes. Diabet Med 2012; 29:937–944.

22 Afarideh M, Esteghamati VZ, Ganji M, Heidari B, Esteghamati S, Lavasani S, *et al.* 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. Can J Diabetes 2019; 43:336–344.

23 Celikbilek A, Akyol L, Sabah S, Tanik N, Adam M, Celikbilek M, *et al.* S100B as a glial cell marker in diabetic peripheral neuropathy. Neurosci Lett 2014; 558:53–57.

24 Jamal P, Kazim TJ, Banna H. Assessment of the S100 protein of the common peroneal nerves in diabetic patients. Diyala J Pure Sci 2010; 6:126–133.

25 Kles KA, Vinik AI. Pathophysiology and treatment of diabetic peripheral neuropathy: the case for diabetic neurovascular function as an essential component. Curr Diabetes Rev 2006; 2:131–145.

26 Leinninger GM, Edwards JL, Lipshaw MJ, Feldman EL. Mechanisms of disease: mitochondria as new therapeutic targets in diabetic neuropathy. Nat Clin Pract Neurol 2006; 2:620–628.

27 Rushmore TH, Morton MR, Pickett CB. The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. J Biol Chem 1991; 266:11632–11639.

28 Raghunath A, Sundarraj K, Nagarajan R, Arfuso F, Bian J, Kumar AP, *et al.* Antioxidant response elements: discovery, classes, regulation and potential applications. Redox Biol 2018; 17:297–314.

29 Filipek A, Jastrzebska B, Nowotny M, Kuznicki J. CacyBP/SIP, a calcyclin and Siah-1- interacting protein, binds EF-hand proteins of the S100 family. J Biol Chem 2002; 277:28848–28852.

30 Zhang Q, Zhou T, Xu X, Guo Y, Zhao Z, Zhu M, *et al.* Downregulation of placental S100P is associated with cadmium exposure in Guiyu, an e-waste recycling town in China. Sci Total Environ 2011; 410–411:53–58.