International Journal of Diabetes in Developing Countries (December 2022) 42 (4):735–740
https://doi.org/10.1007/s13410-021-01029-0

ORIGINAL ARTICLE

Selenoprotein P levels in patients with diabetes mellitus with complications

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Received: 12 May 2021 / Accepted: 24 October 2021 / Published online: 12 November 2021

Abstract

Aims Increasing evidence has shown that selenoprotein P levels are elevated in type 2 diabetes mellitus and are associated with insulin resistance and release. This study aimed to determine if there was a connection between selenoprotein P levels and metabolic parameters in patients with diabetes with microvascular complications.

Methods Serum selenoprotein P concentrations were measured by ELISA in 44 patients with diabetes with complications and 36 patients with diabetes without complications.

Results There was no statistically significant difference in selenoprotein P levels between the groups [1.9 (0.9–2.6) and 1.9 (0.8–2.4) ng/mL, respectively, $p = 0.565$]. Selenoprotein P, glucose, glycosylated hemoglobin, C-reactive protein, triglycerides, total cholesterol, and low-density lipoprotein cholesterol levels were not statistically significantly correlated in patients with complications. However, there was a significant correlation with high-density lipoprotein cholesterol ($r = -0.401$, $p = 0.042$).

Conclusions We did not find high selenoprotein P levels in patients with complications, but its inverse association with high-density lipoprotein cholesterol indicates that it may play a role in developing cardiovascular disease in this community of patients.

Keywords Selenoprotein P · Microvascular complications · Diabetic complications · Diabetes mellitus

Introduction

Diabetes mellitus (DM), which has a mortality rate of about 4.2 million people globally, affects over 500 million people aged 20 to 79 years. It is expected to impact more than 750 million people in the next 25 years [1]. Diabetes, which is considered an epidemic, will continue to be a significant health and economic challenges both now and in the future as industrialization and food habits improve. The most common form of diabetes is included in the physiopathology of insulin resistance in the tissues in type 2 diabetes mellitus (T2DM). Hyperglycemia caused by insulin resistance can lead to micro- and macrovascular complications. Microvascular complications, such as nephropathy, neuropathy, retinopathy, and ischemia, account for the majority of complications and are associated with morbidity and mortality [2]. Effective treatments and hyperglycemia-controlling diets can dramatically reduce the risk of complications because the complications are usually permanent. Although such effective treatments can reduce microvascular complications by 50–76% [3], they may increase mortality by causing adverse events such as hypoglycemia [4].

The liver releases a group of proteins called hepatokines into the circulation, which affect carbohydrate and lipid metabolism. There is evidence that hepatokines play a role in T2DM [5]. Selenoprotein P (SeP; encoded by SELENOP) is a hepatokine mainly produced by the liver. SeP, also used as a diagnostic parameter for blood selenium status, functions as a selenium supply protein in the body [5–7]. The high level of SeP in patients with diabetes and the fact that the administration of high concentrations of SeP causes insulin resistance in tissues and a decrease in insulin secretion

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from the pancreas make it a diabetogenic protein [8, 9]. Experimental administration of SeP neutralizing antibodies resulted in improvements in glucose metabolism and insulin resistance and release [9]. It has been reported that initial SeP concentrations are a more reliable test for predicting glucose intolerance that will develop after 4 years compared with diabetes parameters such as HOMA-IR, age, glycosylated hemoglobin (HbA1c), and fasting plasma glucose [10]. It was reported that high SeP levels in patients with DM were also associated with inflammation and atherosclerosis [11].

The fact that SeP causes insulin resistance and reduces pancreatic insulin secretion has made it a possible target for diabetes treatment [8]. If a relationship exists between SeP and diabetes complications, it will contribute to the prevention or treatment of complications. However, we found no studies in the literature indicating a relation between SeP and the complications of diabetes. Therefore, this study aimed to evaluate whether there was a relationship between the microvascular complications of T2DM and SeP.

Methods

Study population

In this cross-sectional study, 44 patients with T2DM with complications, and 36 age- and sex-matched patients with T2DM without complications who presented to the internal diseases outpatient clinic were included. Pregnant women, patients with chronic diseases other than diabetes, and those taking selenium and other supplements for the last 6 months were excluded from the study. All patients were evaluated for diabetic complications through clinical examinations and specific laboratory tests.

Of the patients with complications, 12 had nephropathy, six had neuropathy, seven had retinopathy, and six had ischemia. Twelve patients had more than one complication, six had nephropathy and neuropathy, two had nephropathy and retinopathy, one had retinopathy and ischemia, one had nephropathy, retinopathy and ischemia, one had neuropathy, retinopathy and ischemia, and one person had neuropathy, nephropathy, retinopathy, and ischemia.

Laboratory analysis

Venous blood samples were obtained from all patients from the forearm in the morning after 8–10 h of fasting. Blood was collected in plain tubes and allowed to clot for 30 min before being centrifuged for 10 min at 2000 *g*. Approximately 0.5 mL of the serum was transferred to microcentrifuge tubes and stored at −80 °C until the SeP levels were measured. Routine biochemical tests were immediately performed. HbA1c levels of the blood samples collected in K_2EDTA tubes were studied. Urine albumin, protein, and creatinine tests were performed in the first urine in the morning.

Glucose, creatinine, lipid parameters [low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, total cholesterol, and triglycerides], C-reactive protein (CRP) in serum, creatinine, protein, and albumin in urine were studied in an autoanalyzer (AU5840; Beckman Coulter, CA, USA) using routine laboratory methods. HbA1c was measured using high-performance liquid chromatography (HPLC) (Premier Hb9210; Trinity Biotech, Co. Wicklow, Ireland). Urine protein/creatinine and urine albumin/creatinine ratios were obtained by calculation.

Serum SeP concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Beijing, China) using the sandwich-ELISA method. The test was conducted according to the kit instructions. The optical density was measured spectrophotometrically at 450 nm using a microplate reader (SPECTROstar Nano, BMG Labtech).

Statistical analysis

According to the data distribution, results were expressed as mean ± SD or median (25th–75th percentile). The normality of the data was determined using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Student's *t* test was used for parametric data, and the Mann–Whitney *U* test was used for non-parametric data to compare the two groups. Chi-square analysis was used to compare categorical results. The Kruskal–Wallis test was conducted to compare the differences between more than two groups. The Mann–Whitney *U* test was performed to test the significance of pairwise differences using Bonferroni correction to adjust for multiple comparisons. The relationship between SeP and other parameters was assessed using Spearman's non-parametric correlation test. All statistical analyses were performed using the SPSS for Windows, version 21.0 statistical package (SPSS, Chicago, IL). p values <0.05 were considered statistically significant.

Results

Comparisons of anthropometric measurements and biochemical parameters between the complication group and the without complication group are summarized in Table 1. There were no statistically significant differences in serum SeP levels between the DM groups

Table 1 Laboratory characteristics and anthropometric data of the groups

Parameters	Patients with complication $n=44$	Patients without complication $n=36$	p value
Age	61.9 ± 9.4	59.4 ± 10.7	0.261
Sex, F/M	20/24	20/16	0.369
Waist circumference (cm)	111.5 ± 11.9	105.7 ± 6.9	**0.009**
Waist to hip ratio	0.99 ± 0.07	0.95 ± 0.07	**0.012**
BMI (kg/m^2)	33.5 (29.5-38.5)	32.7 (29.5-36.8)	0.588
Systolic TA (mmHg)	134.5 ± 18.6	133.5 ± 20.4	0.843
Diastolic TA (mmHg)	81.2 ± 11.2	79.1 ± 11.6	0.465
Duration of diabetes (month)	122 (72-195)	120 (48-180)	0.149
SeP (ng/mL)	1.9 (0.9-2.6)	1.9 (0.8-2.4)	0.565
Glucose (mg/dL)	150 (133-195)	146 (118.5-178)	0.287
HbA1c (%)	8.5 ± 1.5	7.8 ± 1.6	**0.038**
Creatinine (mg/dL)	1.0 ± 0.4	0.8 ± 0.2	**0.008**
CRP (mg/dL)	0.4 (0.2-0.7)	0.2 (0.1-0.6)	0.092
Triglycerides (mg/dL)	145 (107-204)	148 (107-216)	0.829
Total cholesterol (mg/dL)	196 ± 45	193 ± 45	0.992
LDL cholesterol (mg/dL)	115 ± 37	109 ± 34	0.596
HDL cholesterol (mg/dL)	44 (40-56)	46 (42-56)	0.253

The values are presented as mean ± SD or median (25th and 75th percentiles). The values in boldface indicate a statistically significant ($p < 0.05$)

BMI, body mass index; *CRP*, C-reactive protein; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *SeP*, selenoprotein P; *TA*, tension arterial

with and without complications. In patients with complications, waist circumference ($p=0.009$), waist-to-hip ratio ($p=0.012$), HbA1c ($p=0.038$) and serum creatinine ($p=0.008$) values were significantly higher than in patients without complications. However, there were no statistically significant differences in diabetes duration, systolic and diastolic blood pressures, body mass index (BMI), and lipid parameters between patients with and without complications.

The results of correlation analysis between SeP and other parameters in the complicated group are summarized in Table 2. Only HDL cholesterol was negatively correlated with SeP ($r=-0.401$, $p=0.042$) (Fig. 1).

Anthropometric measurements and laboratory parameters according to the type of complications are summarized in Table 3. There was no statistically significant difference in SeP values between the patients with and without complications. As expected, urinary protein/creatinine and urine albumin/creatinine ratios were significantly higher in patients with nephropathy than in patients without complications ($p<0.001$, $p<0.001$, respectively). Only the duration of diabetes was significantly longer in patients with retinopathy than in patients without complications ($p=0.003$). There was no statistically significant difference in any parameter in patients with ischemia and neuropathy. Patients with more than one complication had significantly higher HbA1c levels than patients without complications ($p=0.001$). In addition, these patients had significantly higher urine protein/creatinine and albumin/creatinine ($p<0.001$, $p<0.001$, respectively) than patients without complications.

Table 2 Correlation of SeP with other parameters in the patients with complication

Parameters	Correlation coefficient (r)	p value
Duration of diabetes	0.027	0.869
Waist circumference	− 0.251	0.109
Waist to hip ratio	− 0.075	0.639
BMI	− 0.226	0.149
Glucose	0.062	0.690
HbA1c	0.152	0.326
CRP	− 0.333	0.096
Protein/creatinine ratio	0.093	0.612
Albumin/creatinine ratio	0.136	0.377
Triglycerides	0.257	0.205
Total cholesterol	− 0.080	0.699
LDL cholesterol	− 0.112	0.585
HDL cholesterol	− 0.401	**0.042**

See Table 1 for abbreviations. The value in boldface indicate a statistically significant ($p < 0.05$)

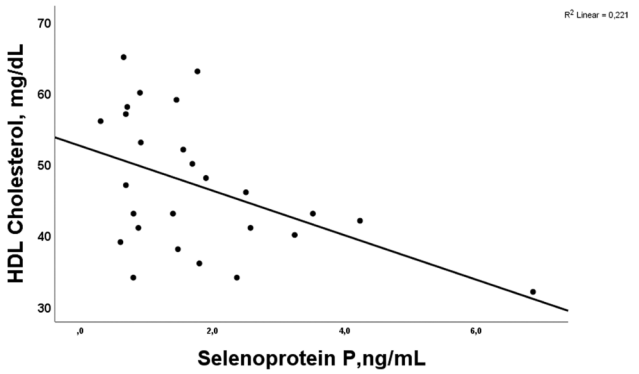

Fig. 1 Scatter plot of HDL cholesterol and SeP in the patients with complication

Discussion

Our aim in the present study was to evaluate whether SeP, which is stated to be diabetogenic, had a relationship with microvascular complications seen in patients with T2DM. Our study revealed that serum SeP concentrations in patients with diabetic with complications were not different from those without complications. As far as we know, it is the first study to examine SeP levels in such a population.

Serum SeP levels are elevated in people with diabetes, and according to both experimental and clinical research, SeP induces insulin resistance [8, 11–13]. Misu et al., in an experimental study, reported that hepatic SeP expression and serum SeP concentrations increased in DM and SeP levels were positively correlated with fasting plasma glucose and HbA1c levels. They also showed that when they gave high doses of SeP to primary hepatocyte cell cultures and mice, SeP increased insulin resistance by disrupting insulin signaling [8]. Zhang et al. showed that serum SeP concentrations were higher in people with uncontrolled diabetes (HbA1c > 7%) in a study of 176 patients with T2DM and 142 healthy people [13]. Unlike this study, serum SeP levels in the complicated group with uncontrolled diabetes were comparable to those in the patients without complications in our study. Yang et al. found that serum SeP concentrations increased in proportion to the disease status in three groups of 100 individuals, including non-DM, prediabetes, and DM groups [11]. However, in their study, SeP concentrations were not different between patients with prediabetes and those who developed diabetes. Our study found no difference in SeP concentrations in the complication phase, the more advanced form of diabetes, which supports this viewpoint.

There are also studies in the literature reporting that serum SeP is not associated with diabetes. Altınova et al. showed that SeP levels in pregnant women with gestational diabetes were not different from healthy and non-pregnant women [14]. Another study found that SeP did not differ between healthy and people with T2DM in a small number of study groups [15].

High HbA1c is a valuable marker for DM diagnosis and monitoring, indicating insufficient glucose control for at least 2-3 months. High HbA1c levels are also associated with micro and macrovascular complications and mortality in DM [16]. The literature on the relationship between HbA1c and SeP is contradictory, with studies finding positive [8] and negative [15] correlations, as well as studies finding no association [14]. In our study, HbA1c levels were higher in the complicated group as expected, but there was no correlation between HbA1c and SeP levels.

Dyslipidemia is common in diabetes. High triglycerides, cholesterol, and low HDL levels play an essential role in developing atherosclerosis in individuals with diabetes [17]. Although the precise cause of low HDL in diabetes is unknown, it is thought to be caused by an increase in HDL catabolism due to insulin resistance [18]. In our study, patients with complications had lower HDL levels, and there was a negative correlation between SeP and HDL. However, there was no correlation between SeP and HDL in patients without complications (data not shown). This inverse relationship between SeP and HDL in the complicated group suggests that elevated SeP levels may be associated with an increased risk of cardiovascular disease, common in people with diabetes. In contrast to our results, in a large prospective study, Schomburg et al. [19] reported that low SeP levels were a predictor of cardiovascular disease and death. They attributed this to factors other than HDL, such as the antioxidant property of SeP. Furthermore, only about 11% of the participants in their study had diabetes.

Our study has several limitations. Apart from the low number of patients in the complications group, the presence of metformin-receiving patients in the study groups could be confusing because metformin inhibits the expression of SeP mRNA in the liver, lowering SeP levels [20]. Thus, SeP levels in patients who received metformin treatment may have been affected. Although it is not well established if feeding low or high in selenium may lead to various pathological conditions, in our study, the dietary habits of the patients were not questionned. Another thing to consider is the lack of detailed validation of the SeP ELISA assay in order to obtain a more accurate level of SeP levels in serum [21].

Our findings indicate that SeP, which has previously been identified as a diabetogenic protein, has no relationship with the microvascular complications of diabetes. However, the inverse correlation between SeP and HDL in patients with diabetes with complications suggests that

Table 3 Anthropometric and laboratory findings of patients by type of complication.

Parameters	Patients without complication ($n=36$)	Patients with nephropathy ($n=13$)	p value	Patients with neuropathy ($n=6$)	p value	Patients with retinopathy ($n=7$)	p value	Patients with ischemia ($n=6$)	p value	Patients with multiple complications ($n=12$)	p value
Waist circumference (cm)	105 ± 6.9	112 ± 14.2	0.150	113 ± 11.4	0.116	111 ± 6.0	0.055	114 ± 13.9	0.152	109 ± 12.0	0.366
Waist to hip ratio	0.95 ± 0.1	0.98 ± 0.1	0.200	1.02 ± 0.1	0.060	0.97 ± 0.1	0.483	0.96 ± 0.1	0.886	1.00 ± 0.1	0.032
BMI (kg/m2)	32.7 (29.5–36.8)	34.7 (26.6–40.0)	0.642	35.0 (33.8–35.7)	0.196	31.0 (29.7–36.7)	0.829	35.2 (30.8–42.7)	0.426	30.3 (29.0–36.7)	0.694
Duration of diabetes (month)	120 (48–180)	60 (18–144)	0.320	120 (60–120)	0.926	240 (240–350)	**0.003**	108 (38–130)	0.796	180 (122–222)	0.010
SeP (ng/mL)	1.9 (0.8–2.4)	2.3 (1.2–2.9)	0.319	1.9 (0.7–2.9)	0.857	2.1 (1.5–2.5)	0.693	1.9 (0.9–2.5)	0.653	1.6 (0.8–2.4)	0.739
Glucose (mg/dL)	146 (119–178)	148 (141–210)	0.217	144 (123–154)	0.999	113 (105–176)	0.366	133 (110–147)	0.235	187 (166–250)	0.006
HbA1c (%)	7.8 ± 1.6	8.3 ± 1.5	0.153	8.3 ± 1.6	0.577	8.1 ± 1.5	0.554	7.9 ± 0.9	0.439	9.3 ± 1.3	**0.001**
Creatinine (mg/dL)	0.8 ± 0.2	1.1 ± 0.4	0.013	1.3 ± 0.9	0.042	0.9 ± 0.2	0.147	0.9 ± 0.1	0.408	0.9 ± 0.3	0.482
CRP (mg/dL)	0.2 (0.1–0.6)	0.3 (0.3–0.4)	0.436	0.7 (0.6–1.3)	0.159	0.4 (0.3–0.6)	0.479	0.3 (0.2–0.4)	0.856	0.7 (0.3-1.6)	0.042
Protein/creatinine ratio (mg/g)	96 (77–117)	216 (158–951)	**< 0.001**	64 (53–96)	0.111	89 (75–109)	0.732	98 (77–117)	0.874	382 (197–460)	**< 0.001**
Albumin/creatinine ratio (mg/g)	8.9 (6.5–15.5)	98.7 (34.2–182.6)	**< 0.001**	3.7 (3.4–13.3)	0.196	13.8 (8.1–28.6)	0.308	13.6 (4.7–17.3)	0.640	152.8 (41.8–224.1)	**< 0.001**

See Table 1 for abbreviations

The values are presented as mean ± SD or median (25th and 75th percentiles)

Data for each type of complication were compared statistically to patients without complications. The values in boldface indicate a statistically significant ($p < 0.003$)

SeP may play a role in the development of cardiovascular disease in patients with diabetes, but this relationship should be evaluated with more comprehensive studies.

Acknowledgements The authors would like to thank the Scientific Research Projects Unit of Kırşehir Ahi Evran University.

Funding This study was supported by Kırsehir Ahi Evran University Scientific Research Projects Unit.

Data Availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval Kırsehir Ahi Evran University Faculty of Medicine approved the study of Medicine Ethics Committee (approval date and number: 2020-04/31). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication All of the authors confirm the publication.

Conflict of interest/competing interests The authors declare no competing interests.

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