**STROBE Statement—**

**Title and abstract**

The relationship between diabetic polyneuropathy, serum visfatin, and oxidative stress biomarkers

 Diabetic polyneuropathy is a common complication of diabetes. We investigated 392 patients with diabetes for neuropathy clinical signs using two validated screening methods; Subjective Peripheral Neuropathy Screen Questionnaire (SPNSQ) and Michigan Neuropathy Screening Instrument (MNSI). For all patients, oxidative stress status, visfatin, and thiol-disulfide balance were analyzed Neuropathy findings were observed 43.9% of the patients but neuropathy related symptoms were reported only 20.7% of the patients. Serum glucose, Hba1c, and visfatin were positively correlated with each other and total oxidative stress index. Total and native thiol had a negative directed correlation with oxidative status. And also between visfatin and total with native thiol negative correlation was observed (p<0.005, r= -0,338), (p<0.005, r= -0,448). In patients with diabetes, neuropathy is one of the major complications. Oxidative status has an important place in pathogenesis, and the availability of visfatin and thiol-disulfide balance are the subjects that need to research.

**Introduction**

 **2 Background/rationale**

 Diabetic Polyneuropathy is the most common complication along with a 50% lifetime prevalence in patients with diabetes. Clinic evaluation alone is insufficient for exclusion because of absence of the positive symptoms. Diagnostic guidelines based on specific examination methods that is not always easy to apply, and a practical method or a biochemical parameter is exciting for all physicians that deal with these patients.

**3 Objectives**

 In this study we wanted to investigate the sensitivity and availability of a protein –visfatin- in clinical practice that unstudied before. At the same time, we wanted to evaluate its oxidative or antioxidative location along with thiol-disulfide balance.

**Methods**

**4 Study design**

 An observational study was performed.

**5 Setting**

 Previously diagnosed patients with diabetes are included in the study. Our patients with diabetes come to routine control every 3 months with an appointment. Between October 2018 and April 2019, we randomly included this group of patients in the study with informed consent. A total of 392 patients whose neuropathy examinations completed were included in the study. It was performed at the Bezmialem Vakif University Internal Medicine and Endocrinology department policlinics. All participant data collection was obtained one time when the patient comes for routine glucose follow up.

**6 Participants**

 The patients with diabetes that can answer the questionnaires and consentient to the informed consent were included. For the selection of participants, we did not use any other sources or methods. Exclusion criteria are as follows; the patients with acute infection or other lymphoproliferative and chronic infections, patients with monoclonal gammopathy, vasculitis, alcoholism, chronic renal failure, sarcoidosis, Sjogren disease, amyloidosis, neoplasms, paraneoplastic syndromes, with a certain diagnosis for hereditary, demyelinating or multifocal neuropathies, radiculopathy, mononeuritis, and cerebrovascular diseases.

**7 Variables**

Because of subjective properties for questionnaires, neuropathy clinical symptoms can be evaluated with missing information. Also, all of the patients were not examined for neuropathy clinical signs by the same physician along with a minor effect on a patient basis.

 Complaints of the patients were evaluated using the SPNSQ and MNSI. SPNSQ contains 15 questions about the symptoms of neuropathy. The total score is obtained by counting the answers of yes. The sum of the scores range from 0 to 15 and determine the cases from no neuropathic symptoms to the severe neuropathic symptoms. MNSI questionnaire contains 15 ‘yes/no’ questions regarding neuropathy. For the questions 1–3, 5–6, 8–9, 11–12, 14–15, ‘Yes’ answer and for questions 7 and 13 ‘ No’ answer was scored as one point. Questions 4 and 10 are not included in the published scoring algorithm. The sum of the questionnaire score of 7≥ was accepted as abnormal. MNSI examination was performed by the physicians participating in the study. The total MNSI score was pointed over 10 points and the score ≥ 2.5 was accepted as abnormal.

**8 Data sources/ measurements**

 The existence of neuropathy clinic symptoms and signs evaluated. Serum glucose, glycosylated hemoglobin (HbA1c), serum triglycerides, low-density lipoprotein- cholesterol (LDL-C), serum creatinine, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), vitamin B12 and serum visfatin were tested using with standard commercial assay kits. Serum total oxidant and antioxidant status were analyzed. The oxidative stress index was calculated. Thiol-disulfide homeostasis was calculated along with the standardized method using the total and native thiol measurements.

**9 Bias**

The predictive effect of the studied parameters can be discussed, but we believe that effective results will be obtained with large population-based case-control studies.

**10 Study size**

 This study was carried out at Bezmialem Vakıf University Medical Faculty, which serves a large number of patient groups. Patients with diabetes who met the inclusion criteria and applied to the hospital on the specified dates were included in the study.

**11 Quantitative variables**

 All of the quantitative variables were correlated with each other. Only for MNSI neuropathy questionnaire and examination, cutting points specified in the literature were applied and it was presented properly in the article.

**12 Statistical methods**

 For all evaluations; in terms of categorical data, the chi-square test, and the cuts of averages, the independent sample t-test was used. The Pearson correlation coefficient was calculated for the relationship between all variables. p<0.05 was accepted as significant.

**Results**

**13 Participants**

 The patients who applied to the outpatient clinic were included in the study. The data of the patients who answered the questionnaire appropriately, whose examination could be completed, blood samples were taken and stored appropriately were analyzed. As a result, the data of 392 patients were reliably evaluated.

**14 Descriptive data**

Study participants were almost in the same demographic and social status. There is no potential confounders. There are no follow uptime.

**15 Outcome data**

There is no additional outcome data.

**16 Main results**

The mean age of the patients was 57.5±9 years. The mean disease duration was 12±7.29 years. SPNSQ, MNSI questionnaire, and MNSI exam scores were correlated with each other (*p*<0.005). Between the disease duration and SPNSQ, MNSI questionnaire and exam significant positive correlation was observed (*p*<0.005, r=0.275*, p*<0.005, r=0.242, *p*=0.027, r=0.119). Visfatin was positively correlated with higher glucose, Hba1c, total oxidant status and oxidative stress index (p<0.005, r=0,537, r=0,753, r=0,407, r=0,587). But it was negatively correlated with total antioxidant status (r= - 0,499). Total with native thiol was negatively correlated with glucose, Hba1c, total oxidant status, and oxidative stress index but they were positively correlated with total antioxidant status. Statistically significant negative directed correlation was observed between visfatin and total with native thiol *(p*<0.005, r= - 0,338), (p<0.005, r= - 0,448).

**17 Other analyses**

There is no other analyses.

**Discussion**

**18 Key results**

Because of subjective property, the sensitivity and reliability of questionnaires are insufficient for neuropathy screening in patients with diabetes. -Agathos E, Tentolouris A, Eleftheriadou I, et al. Effect of α-lipoic acid on symptoms and quality of life in patients with painful diabetic neuropathy *J Int Med Res*. 2018-

Poor glycemic control is strongly related to microvascular complications that including diabetic neuropathy. -Khawaja N, Abu-Shennar J, Saleh M, et al. The prevalence and risk factors of peripheral neuropathy among patients with type 2 diabetes mellitus; the case of Jordan Diabetol Metab Syndr. 2018-

Increased oxidative/nitrosative stress is one of the major determinants in neuropathy pathogenesis. -Callaghan BC, Cheng HT, Stables CL, et al. Diabetic neuropathy: clinical manifestations and current treatments. Lancet Neurol. 2012-

The usefulness of the markers associated with an oxidative balance is one of the major research topics. Visfatin and thiol-disulfide status are between inspiring parameters.- Indulekha K, Surendar J, Anjana RM et al. Metabolic obesity, adipocytokines, and inflammatory markers in Asian Indians--CURES-124. Diabetes Technol Ther. 2015-, -Ergin M, Aydin C, Yurt EF, et al. The Variation of Disulfides in the Progression of Type 2 Diabetes Mellitus. Exp Clin Endocrinol Diabetes. 2018-

**19 Limitations**

 It would be better to evaluate neuropathy clinical signs with more sensitive methods like electrophysiological examination.

 Missing medication history is one of our deficiencies since additional diagnoses of the patients and the drugs used may affect our results.

 Detailed knowledge of antidiabetic treatment used could provide additional predictions when interpreting the results.

**20 Interpretation**

In this study, first of all, we wanted to evaluate and find out a useful method for diabetic polyneuropathy early diagnosis. We saw that many studies with biochemical markers were presented in the literature. But in clinical practice, none of them were used as a routine diagnostic method for different reasons For this purpose we wanted to study visfatin protein on human subjects first in the literature. Also in blood samples, we tested thiol-disulfide balance and oxidative balance along with visfatin, and significant correlations were obtained. For thio-disulfide balance, our results were compatible with other studies that included patients with diabetes. The limitations of the study include insufficient knowledge about medications and detailed evaluations of the patients.

**21 Generalisability**

 Our study showed that there is a need for a practical and high-sensitive tool for early diagnosis of neuropathy in clinical practice. Biochemical markers may seem to be the most practical method, but it is unlikely that many of them will be usable because they are not specific to the pathogenesis involved. Among this thiol-disulfide balance may be cost- effective, and visfatin may be a high predictive marker for early diagnosis. However, more comprehensive studies are needed.

**Other information**

**22 Funding**

 Bezmialem Scientific Research Projects Unit is partly funded by the biochemical kits that used for our study. Other data were analyzed in the biochemistry laboratories of our hospital partially with personal financial facilities.