Proinflammatory cytokines predict the incidence of diabetic peripheral neuropathy over 5 years in Chinese type 2 diabetes patients: A prospective cohort study

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ABSTRACT

Background: Inflammation has been implicated in the pathogenesis of diabetic peripheral neuropathy (DPN) as suggested in various cross-sectional studies. However, more convincing prospective studies in diabetes patients are scarce. Therefore, we aimed to evaluate whether proinflammatory cytokines could predict the incidence of DPN through a prospective study with a five-year follow-up.

Methods: We followed up 315 patients with diabetes who did not have DPN, recruited from five community health centers in Shanghai in 2014, for an average of 5.06 years. Based on the integrity of blood samples, 106 patients were selected to obtain the proinflammatory cytokines. Plasma markers of proinflammatory cytokines at baseline included interleukin-6 (IL-6), tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), vascular endothelial growth factor (VEGF), and intercellular adhesion molecule 1 (ICAM-1). Neuropathy was assessed by MSNI at baseline and during follow-up.

Findings: Among the 106 chosen patients, 63 developed DPN after 5.06±1.14 years of follow-up. The baseline plasma levels of TNF-\(\alpha\), IL-6, and ICAM-1 were higher in the neuropathic group (p<0.05). In multivariate models, increased plasma levels of TNF-\(\alpha\) (hazard ratio, HR: 8.74 [95% confidence interval, CI: 1.05 – 72.68]; p<0.05) and ICAM-1 (HR 23.74 [95% CI: 1.47 – 383.81]; p<0.05) were both associated with incident DPN, after adjusting for known DPN risk factors.

Interpretation: Increased plasma levels of proinflammatory factors, especially TNF-\(\alpha\) and ICAM-1, predicted the incidence of DPN over 5 years in Chinese diabetes patients, but larger longitudinal studies are required for confirmation.

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1. Introduction

Diabetic peripheral neuropathy (DPN) is the most common diabetic microvascular complication, although the incidence and prevalence of DPN vary greatly among epidemiological studies [1,2]. The most frequent form, distal sensorimotor polyneuropathy (DSPN), affects approximately 30% of people with diabetes, while the incidence of DSPN is nearly 2% per year [3]. As the prevalence of diabetes increases, the prevalence and morbidity of DPN will also increase [4]. Despite the high disability and mortality rates of DPN, only few efficient therapeutic agents are available. Thus, early prediction and
diagnosis, enabling early intervention to delay or prevent negative consequences related to DPN, are especially important.

Evidence that systemic inflammation plays an essential role in the pathogenesis of DPN is emerging from both experimental and clinical studies. Immune cells including CD4+ T cells, CD8+ T cells, macrophages, and mast cells have already been detected in injured nerves of patients with DPN [5, 6]. Our previous study showed that interleukin-1 receptor antagonist (IL-1RA), which could inhibit inflammation, delayed the development of DPN and had a protective effect on DPN in animal models [7]. We also reported that the neutrophil-to-lymphocyte ratio (NLR), an indicator of systemic inflammation, was associated with the status of DPN even after adjusting for potential related factors [8]. However, most clinical studies available to date have been cross-sectional [9], and data from the Chinese population are still lacking. Therefore, this follow-up study was conducted with an aim to analyze whether plasma proinflammatory cytokines could predict the incidence of DPN over five years.

2. Methods

2.1. Study design and patients

In this prospective follow-up study, we aimed to examine whether proinflammatory cytokines were associated with the incidence of DPN. All participants were recruited from five community hospitals in Shanghai, People’s Republic of China, in 2014 and followed up until 2019. Inclusion criteria included (1) willingness to participate in the study, (2) diagnosis of type 2 diabetes according to the 1999 World Health Organization diagnostic criteria, (3) age ≥18 years, and (4) valid Michigan Neuropathy Screening Instrument (MNSI) score. Exclusion criteria included (1) peripheral neuropathy other than diabetic origin, (2) pregnancy, (3) coincident major psychiatric disorders, (4) neurological diseases, and (5) diagnosis of DPN.

All examinations were performed by trained doctors and nurses.

2.2. Ethics

The study was approved by the Human Investigation Ethics Committee of Huashan Hospital. With a full understanding of the study, each participant signed the informed consent form voluntarily.

2.3. Demographic and clinical data

All participants completed a questionnaire to obtain information on demographic and clinical data, including age, sex, duration of diabetes, current medications, and smoking and alcohol history. Questionnaire completion was followed by a brief but standardized clinical examination involving height, weight, waist circumference, hip circumference, and blood pressure. Waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²).

2.4. Laboratory data

A fasting venous blood sample was collected from each participant. Levels of total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), serum creatinine (Scr), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were analyzed using an automatic analyzer (Hitachi 7600 chemical analyzer). Levels of glycated hemoglobin (HbA1c) were estimated by high-pressure liquid chromatography using an analyzer (HLC-723G7, Tosoh Corporation, Japan). The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation developed for the Chinese population. The levels of glucose, C-peptide, and insulin were measured after overnight fasting and 120 min after a 100-g steamed bread meal test, to avoid severe glucose fluctuation in patients with type 2 diabetes. Fasting plasma glucose (FPG) and postprandial plasma glucose (PPG) were analyzed using the hexokinase method and an automatic analyzer (Hitachi 7600 chemical analyzer). C-peptide and insulin levels were measured using the chemiluminescence method with an ADVIA Centaur XP automatic analyzer (Siemens Healthcare Diagnostics). The urinary albumin-to-creatinine ratio (ACR) was measured by an enzymatic method on the Roche/Hitachi cobas c system (Roche Diagnostic GmbH, Mannheim, Germany).

2.5. Proinflammatory cytokines

Proinflammatory cytokines including TNF-α, IL-6, ICAM-1 as well as VEGF were quantified using a Luminex 200 apparatus (Luminex Corp.) and Millipore multiplex immunoassay panels (Millipore).

2.6. Assessment of DPN

Neurological examination was performed using the MNSI assessment. The assessment evaluated each abnormality revealed in foot appearance (deformities, dry skin, callus, infection, or fissure), foot ulceration, ankle reflex presence, and vibratory threshold by tuning fork. Responses were assigned scores of 1 (absent), 0.5 (decreased), or 0 (normal) for each foot (maximum score = 8). The standard score for the diagnosis of DPN was >2 points as previously suggested in literature [10, 11].

2.7. Statistical analysis

All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). All data were checked for normality before analysis, and they are presented as means ± SD for normally distributed variables and median, 25th percentile, and 75th percentile (Median [P25, P75]) for nonnormally distributed and continuous variables. Both anthropometry and biochemical measurements were compared between groups, using Student’s t-test for normally distributed continuous data and nonparametric tests for variables without a normal distribution. Categorical variables were presented as frequencies and proportions and analyzed using the $\chi^2$ test or
Fisher’s exact test. Multivariate logistic regression analysis was performed to evaluate the association between DPN and proinflammatory cytokines after adjusting for other variables. P values of less than 0.05 were considered to indicate statistical significance for all analyses.

2.8. Role of the funding

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors (Bin Lu, Shuo Zhang) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

3.1. Study participants

A total of 476 diabetic patients were evaluated for eligibility in 2014, and 161 patients were excluded due to a diagnosis of DPN. After follow-up for 5.06±1.14 years, the remaining 315 participants were divided into the non-DPN group (N = 152) and the DPN group (N = 163) according to their MNSI examination scores, which were evaluated in 2019 at follow-up (Table 1). Among 315 patients without DPN, the blood samples were collected based on participants’ informed preference. 209 patients (82.3%) out DPN, the blood samples were collected based on participants’ informed preference. 209 patients (82.3%) out DPN, the blood samples were collected based on participants’ informed preference. 209 patients (82.3%) out DPN, the blood samples were collected based on participants’ informed preference. 209 patients (82.3%) out DPN, the blood samples were collected based on participants’ informed preference.
three cytokines was significantly higher. However, there was no significant difference in the incidence of DPN between groups graded according to the tertiles of VEGF (Fig. 4).

### 3.4. TNF-α and ICAM-1 levels were significantly associated with the incidence of DPN

The multiple logistic regression model shown in Table 4 demonstrates that plasma levels of TNF-α and ICAM-1 at baseline were significantly associated with the incidence of DPN at follow-up (first tertile vs. second-third tertile), even after adjusting for age, sex, BMI, duration of diabetes, HbA1c, status of dyslipidemia, and hypertension, status of smoking or drinking, physical activity and used of nonsteroidal anti-inflammatory agents at baseline.

### 4. Discussion

Multiple well-designed but cross-sectional studies have demonstrated higher systemic levels of proinflammatory biomarkers in patients with DPN than in those without DPN. Only a few prospective studies have sought to identify biomarkers that are linked with the incidence of DPN. Herder et al. conducted a population-based study in older adults of European descent, which suggested that TNF-α and IL-6 were predictors of incident distal sensorimotor polyneuropathy (DSPN), whereas IL-1RA and ICAM-1 were related to the progression of DSPN [12]. The study [12] mainly focused on the European population and included both prediabetic and diabetic patients. However, data on the Chinese population are still lacking. Another prospective study investigated the potential role of cell adhesion molecules in the pathogenesis of DPN [19]. The sample size of that study was relatively small (28 patients), and pro-inflammatory cytokines were not included. In our prospective study, in patients with type 2 diabetes, we found that elderly Chinese patients who developed DPN had higher levels of TNF-α, IL-6, and ICAM-1 at baseline. Interestingly, baseline VEGF levels in the group that had developed DPN at follow-up tended to be higher than that in the group without DPN. After adjusting for confounding factors, the association between IL-6 and the incidence of DPN was attenuated, while higher levels of TNF-α and ICAM-1 remained related to the incidence of DPN. It is worth mentioning that there were no marked between-group differences among 106 selected patients with known risk factors, such as glucose control, duration of diabetes, and blood pressure, probably due to the reduced sample size (Table 2). Even so, the differences in the pro-inflammatory cytokines between groups remained significant.

TNF-α is a monocyte-derived cytotoxin that may induce directed inflammatory responses [13]. As a critical regulator of immune function, TNF-α mainly binds to its receptor-TNF-α receptor 1 (TNFR1) [14]. TNF-α and TNF-α-related signaling can exacerbate vessel inflammation and oxidative stress occurring in type 2 diabetes [15], which can further contribute to the development of DPN [16]. In addition, a cross-sectional study based on observational data indicated that the TNF-α system was also activated in participants with type 1 diabetes and DPN, regardless of glycemic control and insulin resistance [17]. Due to the potential therapeutic value of TNF-α, several experimental studies have been performed. One study reported that the inhibition of TNF-α in rats with DPN using recombinant human TNFR-antibody fusion protein could relieve nerve injuries more effectively than that in untreated rats with DPN [18]. Thus, there is an experimental basis to support the predictive value of TNF-α over-activation in patients with DPN.
Elevated levels of VEGF and ICAM-1 were both biomarkers of endothelial dysfunction. In regard to ICAM-1, the results of the present study were consistent with those of previous studies. A five-year prospective study involving 28 diabetic patients suggested that ICAM-1 was strongly correlated with peroneal nerve conduction velocity and might be a predictor of the development of DPN [19]. Matthieu Roustit et al. launched a cross-sectional analysis and found that ICAM-1 was significantly associated with DPN, due to their higher neuropathy disability scores [20]. However, no significant difference were observed between two groups in VEGF at the present study, in line with the conclusion arrived by Doupis J [21], indicating the controversial effect of VEGF on the development of DPN.

Whether IL-6 is a friend or a foe for DPN is not yet clear. TNF-α can stimulate mononuclear cells secreting cytokines, including IL-6, and both TNF-α and IL-6 contribute to the development of DPN [22]. Nevertheless, one experimental study demonstrated that IL-6 treatment improved motor and sensory nerve conduction velocity, allodynia, and thermal hyperalgesia in experimental diabetes [23]. The intricate role of IL-6 may explain the discrepancy between the results of Herder et al. [12] and those of ours.

The main limitation of the present study is the lack of peripheral neurophysiologic tests or some other objective examinations, like thermal thresholds and corneal confocal microscopy for the diagnosis of DPN. However, the MNSI assessment is sufficient to satisfactorily characterize the research cohort. In addition, the extrapolation of the conclusions of our study is limited because only Chinese elderly type 2 diabetes patients were enrolled. Another limitation is the relatively small sample size. Thus, larger scale clinical trials, which include different age groups and various ethnic groups, are required to further validate the conclusions.

In conclusion, this prospective five-year study showed that elevated levels of proinflammatory cytokines could emerge as a useful indicator for predicting the incidence of DPN, which should be confirmed in larger sample studies. We previously reported that injection of IL-1RA improved ultrastructural injuries of diabetic nerves, indicating that early intervention was effective [7]. Based on the results of the present study, early detection of plasma levels of proinflammatory cytokines, especially TNF-α and ICAM-1, may help predict the earliest stages of DPN, enabling early intervention.

Data availability

The data are available on request from the authors.

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Contributions

Hangping Zheng and Wanwan Sun wrote the manuscript. Yuanpin Zhang, Lijin Ji and Qi Zhang collected the data. Xiaoming Zhu and Bin Lu conducted the study and manuscript. Hangping Zheng and Wanwan Sun revised the study and manuscript. Bin Lu and Shuo Zhang conducted the study design and quality control. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2020.100649.

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