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Serum matrix metalloproteases-2 and chemokine ligand-2 levels in patients with painful diabetic neuropathy

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ABSTRACT

Background and objectives. Diabetic neuropathy is a prevalent complication in individuals with type 2 diabetes mellitus (DM). The significance of Matrix metalloproteinase-2 (MMP-2) and chemokine (C-C motif) ligand 2 (CCL-2) neuropeptides in diabetic neuropathy remains largely unexplored. Consequently, we investigated the serum MMP-2 and CCL-2 levels across patients with type 2 DM, with and without neuropathic pain.

Materials and methods. This research was an analytical observational study involving forty participants diagnosed with type 2 DM. The presence of diabetic neuropathy was assessed by DN4 questionnaire. The serum concentrations of MMP-2 and CCL-2 were assessed by the ELISA. An area under the curve analysis was conducted to ascertain the cut-off values for serum MMP-2 and CCL-2 levels.

Results. Mean levels for MMP-2 (278.82±448.64 ng/mL vs. 141.42± 43.43 ng/mL, p=0.218) and CCL-2 (107.54 ± 222.25 ng/mL vs. 39.09 ± 9.05 ng/mL, p=0.182) were higher in the cases than

the control group but not statistically significant. There was a significant difference between groups with a duration of diabetes of more than 3 years (75% vs. 25%, p=0.004). Logistic regression showed that patients with history of diabetes less than 3 years had a lesser risk of painful diabetic neuropathy (OR:0.11, p=0.03).

Conclusions. Serum levels of MMP-2 and CCL-2 were elevated in patients with diabetic neuropathic pain compared to those without neuropathic pain in type 2 diabetes mellitus. However, further trials with larger subjects and prospective design are necessary to extend the findings of our study.

Keywords: Diabetes, neuropathy, MMP-2, CCL-2, neuroinflammation

INTRODUCTION

Diabetic neuropathy is a frequent consequence among individuals with type 2 diabetes mellitus, affecting about 50% of those affected during their lifetime. This illness is defined by a clinical state resulting from localized and diffuse damage to the peripheral and autonomic nerves within the somatosensory system. Neuropathic pain was the primary symptom impacting the sleep cycle, mental health, and general deterioration in the patient's quality of life [1,2]. Recent papers indicated that nerve inflammation plays a pivotal role in the etiology of neuropathic pain in type 2 diabetes, including pro-inflammatory cytokines, enzymes, and the nervous system [3].

Matrix metalloproteinases (MMPs) are a class of endopeptidases that have a role in neuroinflammation by activating proinflammatory cytokines and chemokines. There were over 20 members of MMP peptides. MMP-9 and MMP-2 are two identified sub-endopeptidases predominantly located in the extracellular matrix, cerebrospinal fluid, and serum. Prior research has shown that the onset of neuropathic pain in both early and late stages necessitates MMP-2. Laboratory studies indicate that the inactivation of MMP-2 is pivotal in the pathogenesis of diabetic neuropathy due to myelin irregularities in peripheral nerves and the activation of glial

cells [4]. Nevertheless, limited information exists concerning the human data of this investigation.

Conversely, the stimulation of neuroinflammation in diabetic neuropathy induces the expression of chemokine (C-C motif) ligand 2 (CCL2), a protein that plays multifaceted functions in inflammation-related diseases. This chemokine is secreted from synaptic vesicles in the spinal cord within the dorsal root ganglia, thereby stimulating glial cells and inducing proinflammatory cytokines [5]. Previous studies assessing MMP-9 and CCL2 levels were limited, and the evidence required greater consistency, particularly in human data. This study seeks to investigate the disparity in serum MMP-9 and CCL2 concentrations in individuals with painful diabetic neuropathy. We posited that increased serum levels of MMP-9 and CCL2 contribute to the onset of painful neuropathy in patients with type 2 diabetes.

MATERIALS AND METHODS

This research was an analytical observational study with a case-control design. The current study encompassed 40 individuals diagnosed with type 2 diabetes mellitus. The subjects included individuals with painful diabetic neuropathy (DN4 >4), while the control group consisted of patients with non-painful diabetic neuropathy (DN4 ≤4). Participants who met the inclusion and exclusion criteria were consecutively recruited from the neurology and internal medicine clinic at Prof. Dr. I.G.N.G. Ngoerah General Hospital, Denpasar, Bali, between period of July and September 2021.

The inclusion criteria were patients with type 2 diabetes mellitus, aged 40 to 65 years old, and who had 3 to 5 years of disease duration. Initially, the patients were screened with the DN4 questionnaire. Thus, patients were grouped into cases or control groups based on DN4 scores. Serum blood samples were collected from all subjects. Exclusion criteria were patients with a history of systemic disease (e.g., chronic kidney disease, human immunodeficiency virus infection, cancer), entrapment-related neuropathy, drugs or toxins-related neuropathy, and patients with severe mental illness.

The sample extraction requires about 3 mL of venous blood samples. The levels of MMP-2 and CCK-2 in serum were determined by the ELISA method using kits from the Biomerieux washer 470 reader 270. Calibration curves were drawn for each set of the attached standards.

The subjects' clinical characteristics were analyzed using univariate analysis and presented as percentages (%) and means (standard deviation) or median (min-max). Bivariate analysis was performed using the Chi-square test for categorical data. Meanwhile, an independent *t*-test or Mann-Whitney *U* test was used for numerical variables, depending on the normality of data distribution. Area under the curve analysis was performed to determine the cut-off value of serum MMP-2 and CCL-2 levels. Regression logistic was carried out for multivariate analysis. Statistical significance was set at $p < 0.05$. The analysis was performed using version 26.0 of SPSS.

The study was approved by the Ethical Committee of the Medical University of Udayana University (no. 777/UN14.2.2.VII.14/LT/2021). Informed written consent was collected from all participants.

RESULTS

This study was a case-control study design, with 40 subjects with type 2 diabetes recruited consecutively (20 cases and 20 controls). (Table 1) shows the baseline characteristics and the comparison of MMP-2 and CCL-2 levels between groups. Mean levels for MMP-2 (278.82±448.64 ng/mL vs. 141.42± 43.43 ng/mL, p=0.218) and CCL-2 (107.54 ± 222.25 ng/mL vs. 39.09 ± 9.05 ng/mL, p=0.182) were higher in the cases group than the control group but not statistically significant. Bivariate analysis showed a significant difference between the group with a duration of diabetes of more than 3 years (15 [75%] vs. 5 [25%], p=0.004).

Table 1 Subjects Baseline Characteristics

Variable	Cases n(%)	Control n(%)	p-value
Age, years (mean ± SD)	52 ± 7.57	51,3 ± 5,1	0.733
Gender			
Male	11 (55)	10 (50)	1.000
Female	9 (45)	10 (50)	
Education			
No history of education	1 (5)	0 (0)	0.413
Elementary school	6 (30)	4 (20)	
Junior high school	1 (5)	3 (15)	
Senior high school	7 (35)	11 (55)	
Bachelor	4 (20)	1 (5)	
	1 (5)	1 (5)	
BMI (mean ± SD)	25,25 ± 3,62	25,08 ± 4,82	0.900
Hypertension			
Yes	4 (20)	3 (15)	1.000
No	16 (80)	17 (85)	
History of smoking			
Yes	2 (10)	1 (5)	1.000
No	18 (90)	19 (95)	
Diabetes medication			
Insulin	16 (80)	12 (60)	0.377
Oral	3 (15)	7 (35)	
Combination	1 (5)	1 (5)	
HbA1C ± SD	9,73 ± 2,76	8,72 ± 2,70	0.243

Duration of Diabetes			
< 3 years	5 (25)	15 (75)	0.004*
≥ 3 years	15 (75)	5 (25)	
DN4 scores (median, min-max)	5 (4-8)	2 (0-3)	<0.001*
MMP-2 serum levels (mean ± SD)	278.82 ± 448.64	141.42 ± 43.43	0.218
≥ 135 ng/mL	11 (55%)	7 (35%)	0.717
<135 ng/mL	9 (45%)	10 (65%)	
CCL-2 serum levels (mean ± SD)	107.54 ± 222.25	39.09 ± 9.05	0.182
≥ 36 ng/mL	7 (35%)	5 (29.4%)	0.741
<36 ng/mL	13 (65%)	12 (70.6%)	

Table 2 shows the area *under the curve* (AUC) analysis to determine the cut-off value of serum MMP-2 and CCL-2 levels.

Table 2 Area Under the Curve (AUC) analysis of serum levels MMP-2 dan CCL-2

	AUC	**Sig**	**95% CI**	**Sensitivity**	**Specificity**	**Cut-off value**
MMP-2	0.429	0.441	0.247-0.610	0.45	0.65	135 ng/mL
CCL-2	0.491	0.925	0.308-0.674	0.65	0.70	34 ng/mL

Multivariate analysis with logistic regression showed that subjects with history of diabetes less than 3 years was protective factor of painful diabetic neuropathy (OR:0.11, p=0.03) as shown in Table 3.

Table 3 Logistic regression analysis

Variable	OR	CI 95%	p
Duration of Diabetes < 3 years	0.11	0.027-0.465	0.003*

DISCUSSION

Matrix metalloproteinases (MMPs), a class of protease enzymes, are integral to the pathogenesis of diabetic polyneuropathy, causing neuronal injury and extracellular matrix abnormalities that contribute to the condition. The study's results indicate no association between the incidence of diabetic neuropathic pain in persons with type 2 diabetes mellitus and serum concentrations of MMP-2 and CCL-2. However, the majority of patients with type 2 diabetes suffering from diabetic neuropathic pain in this study exhibited elevated serum levels of MMP-2 compared to the control group (55% vs. 35%). This conclusion is corroborated by the observation that the case group's mean serum MMP-2 level was elevated.

Astrocytes in brain tissue and the spinal cord express the MMP-2 protein. Previous studies indicate that in cases of peripheral neuropathy, the nerve tissue does not produce increased levels of MMP-2 during inflammation. The MMP-2 variant -1306C increases the likelihood of diabetic polyneuropathy by 3.4 times compared to controls; however, the single nucleotide polymorphism at the nucleotide position -1306C>T exhibits a protective effect against diabetic polyneuropathy, as reported in a genetic study by Andjelic et al. [6]. Genetic study indicates that MMP-2 elevates the risk of developing diabetic polyneuropathy. This explains the study's findings, which demonstrate that the case group's MMP2 levels are greater.

The structural role of MMP in polyneuropathy, especially in relation to neuropathic pain, is reasonable. Proteases belonging to the metzincin superfamily, or MMP enzymes, possess a zinc ion binding site that functions as the enzyme's activation site. The principal function of MMP is to degrade components of the extracellular matrix. Studies have shown that illnesses such as cancer, inflammation, and arthritis are caused by the dysregulation of this MMP protein [7-9]. MMP is implicated not just in peripheral nerves but also in glial cells, which contribute to peripheral neuropathy. Neuropathic pain is initiated in its early phase by microglial activation and triggered in its late phase by astrocytic activation. MMP-9 affects microglial activation, while MMP-2 affects astrocytic activation [10-12].

Previous studies indicate that neuroinflammation significantly contributes to the genesis of neuropathic pain. Monocyte chemotactic protein-1 (MCP-1), also known as chemokine C–C motif chemokine ligand–2 (CCL2), is a chemokine that binds to its receptor, C–C motif receptor 2 (CCR2), initiating molecular signalling cascades that ultimately lead to peripheral neuropathic pain. The downstream consequences of N-Methyl D Aspartate Receptor (NMDAR) activation are augmented by chemokine CCL2 binding to CCR2, which further alters and activates the CCL2-CCR2-pERK-GluN2B pathway [13]. The pathogenesis of peripheral neuropathic pain attributed to CCL2 is elucidated in a study by Li et al. [14], which indicates that CCL2 in the periphery induces pain through inflammation by modulating neurons in the Dorsal Root Ganglion (DRG), resulting in elevated HCN2 expression and neuronal hyperexcitability [14].

The small sample size of the study is one of its limitations. Due to restrictions on the number of outpatient visits allowed during the COVID-19 pandemic, data collection during this period was hampered in finding sample criteria that met the inclusion and exclusion criteria.

This study indicates that type 2 diabetes mellitus patients experiencing diabetic neuropathic pain exhibited elevated levels of MMP-2 and CCL-2. The study possesses certain limitations, notably the potential for bias about patient adherence to therapy for the underlying illness, which encompasses challenges with blood sugar regulation and complaints of neuropathic pain. Individuals who have undergone symptomatic treatment for diabetic pain may be excluded

from the case group of this study. Consequently, further research using a prospective design can monitor the factors of therapy status on patient outcomes.

Despite our results was insignificant, this study is the first study in Indonesia to assess the comparison of serum levels of MMP-2 and CCL-2 in patients with diabetic neuropathic pain. This study is anticipated to serve as a foundation for subsequent research on the same disease or other situations.

CONCLUSION

Serum MMP-2 and CCL-2 levels were found to be higher in the group of patients with diabetic neuropathic pain in type 2 diabetes mellitus patients compared to the group without neuropathic pain. The increases serum MMP-2 and CCL-2 levels and risk of developing diabetic neuropathy needed be confirmed in further trials.

Conflict of interest: The authors declare that they have no competing interests.

Authors' contributions:

Conceptualization, I.P.E.W. and I.M.O.A; methodology, K.S., C.T., and H.H.; software, H.H.; validation, A.M.G., K.S., and I.P.E.W.; formal analysis, V.O.W. and T.I.P.; investigation, C.T. and H.H.; resources, I.P.E.W.; data curation, C.T., H.H., and T.I.P; writing—original draft preparation, V.O.W. and T.I.P.; writing—review and editing, K.S., I.P.E.W, and A.M.G.; visualization, T.I.P; supervision, I.M.O.A and K.S.; project administration, V.O.W and H.H.; funding acquisition, I.P.E.W. All authors have read and agreed to the published version of the manuscript.

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FIGURES, TABLES AND SCHEMES

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