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RESEARCH

High leukotriene B4 serum levels increase risk of painful diabetic neuropathy among type 2 diabetes mellitus patients

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Abstract

Background: Painful diabetic neuropathy is one of the most common complications of type 2 diabetes mellitus, with approximately 30–50% of people will experience diabetic neuropathy. Chronic hyperglycemia will cause an inflammatory process that will trigger an immune response included leukotrienes. Leukotriene B4 is associated with hemoglobin glycation levels. This study aimed to determine high serum leukotriene B4 levels and other factors as a risk factor for painful diabetic neuropathy in type 2 diabetes mellitus patient.

Results: Forty-two subjects with 22 cases (median age 56.5 ± 4.9 years) and 20 controls (median age 56.5 ± 5.2 years) group were collected. In bivariate analysis, significant factor for high risk PDN was high leukotriene B4 serum level (OR 5.10; 95% CI 1.34–19.4, p 0.014). Meanwhile, insignificant factors were anti-diabetic drugs (OR 2.139; 0.62–7.37; $p = 0.226$), and duration of diabetes mellitus (OR 2.282; 0.56–9.25; $p = 0.315$). Independent risk factor was serum leukotriene B4 levels (OR 5.10; 95% CI 1.336–19.470; $p = 0.017$).

Conclusions: In this study, high leukotriene B4 serum levels increase the risk of painful diabetic neuropathy among type 2 diabetes mellitus. The leukotriene B4 may consider as a potential biomarker for early detection in high risk for PDN and early treatment.

Keywords: Diabetes mellitus, Painful diabetic neuropathy, Leukotriene B4 levels

Background

Diabetes mellitus (DM) is one of the metabolic diseases with the feature of hyperglycemia, caused by the abnormalities in insulin secretion, insulin action, or both [1]. Untreated hyperglycemia for long periods can damage various organs of the body, such as cardiovascular disease, neuropathy, nephropathy, and eye disease [2]. One of the most common complications of DM is diabetic neuropathy [3].

Almost 30–50% of DM patients will suffer neuropathy [4]. The prevalence of populations suffer from Painful Diabetic Neuropathy (PDN) is estimated to be 8–26% [5]. The incidence of PDN is estimated to be 17.8 per 100,000 people per year and increases with age in the United Kingdom [6]. Another literature found that the prevalence of PDN was 30.3%, consisting of 33.1% type 2 diabetes patients and 14.1% type 1 diabetes patients [7]. PDN might lead increase in the morbidity and mortality rate. DPN requires complex management once diagnosed, but beforehand, it should stress the importance of early screening for diagnosis [8].

Inflammatory mediators cause changes in the expression of sodium channels in nerve fibers that cause abnormal activation in sensory neurons. Those cause an increase in pain stimulus that reaches the spinal cord. In addition, the constant accumulation of nerve

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C fibers causes an increase in release from inflammatory mediators such as transcription factors and nuclear factor-kappa B (NF-kB) [9]. Leukotrienes are inflammatory mediators that importantly play roles in acute and chronic inflammatory diseases. Leukotriene B4 (LTB4) that binds to leukotriene receptors (BLT)-1 is able to trigger inflammation, increase cytokine production, phagocytosis and mediate antimicrobial function [10].

Several kinds of research showed that LTB4 might consider as a diagnostic biomarker for inflammation cases of lymphedema, preeclampsia, obstructive pulmonary disease, and diabetic neuropathy [11–14]. However, regarding the authors' knowledge, LTB4 studies as a risk factor for PDN have never been done in Indonesia before. Therefore, the authors are attracted to conducting this research. This study aims to determine that high levels of LTB4 are a risk factor for diabetic neuropathy pain.

Method

This research is an analytic observational study with a case control design conducted in the neurology polyclinic and diabetic center Sanglah General Hospital Denpasar. This research has obtained ethical clearance No:2969/UN14.2.2.VII.14/LP/2019 from Udayana University Faculty of Medicine/Sanglah General Hospital Research Ethics Committee and also has obtained written consent from patients to participate this study.

The sample subjects in this study were patients who were proven to have type 2 DM aged 45–64 years with (case group) or without (control group) PDN who underwent treatment at the neurology and diabetic center of Sanglah Hospital. The PDN in this study was defined as all neuropathic pain conditions suffered by DM patients as evidenced by a DN4 (Douleur Neuropathique 4 Questions) score ≥ 4 and met the inclusion criteria. DN4 is a questionnaire with interviews and medical examinations to screen for the presence of neuropathic pain [15]. All participants who are willing to be sampled in this study were full alert and not under any coercion.

Exclusion criteria in this study were: (1) type 2 DM patients with a diagnosis period of >5 years [to avoid multifactorial causes other than type 2 DM]; (2) history of neuropathy due to other causes such as chronic kidney disease, chronic liver disease, chronic infection [Human Immunodeficiency Virus, leprosy], malignancy, mechanical nerve entrapment [carpal tunnel syndrome, cervical root syndrome]; (3) history of antiretroviral drugs, chemotherapy, estrogen hormone; (4) history of exposure to toxins such as alcohol, pesticides, mercury, organophosphates and lead; (5) obesity; (6) dyslipidemia; and (7) moderate-to-severe depression [BDI(Beck Depression Inventory)-II result ≥ 20]. The BDI-II was performing to exclude possible confounding of subjectively obtained PDN assessments.

All samples that met the inclusion criteria were collecting by convenience sampling technique to reach the expected sample size. With the patient's consent, blood samples for the examination of LTB4 levels were taken and processed using the ELISA method with the cut-off of ≤ 63.5 pg/mL as a norm value [15]. All data were checked for completeness, and responses were coded and entered into the Statistical Package for the Social Sciences (SPSS) software (version 25; SPSS Inc., Armonk, NY, USA) for windows. Then results were tabulated, graphically, and statistically analysed. Descriptive analyses were showed in both groups regarding categorical or continuous variables. Bivariate analysis were made using the Chi-squared test. Meanwhile, multivariate logistic regression model was utilized to evaluate the independent risk factors of PDN. P value ≤ 0.05 was considered significant.
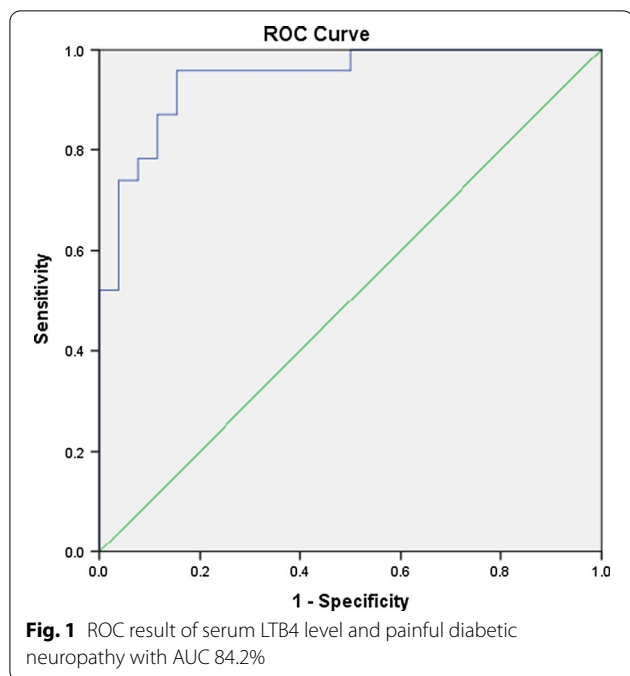
Result

Subjects on both groups were dominated by males ($n = 71.4\%$) and 56.5 years as the median age. As much as 54.8% of subjects used oral anti-diabetic drugs. 71.4% of subjects suffered from diabetes for ≤ 2 years. LTB4 value obtained with a range of 12–124 ng/mL, with a median of 43.80 ng/mL. The characteristics of research subjects from both groups shown in Table 1.

The ROC curve (Fig. 1) shows that the LTB4 value has a pretty good diagnostic value, because the curve is above the 50% line. The AUC value obtained from the ROC method is 84.2% (0.73–0.96%, $p = 0.001$) which statistically indicates sufficient diagnostic strength. The results of the ROC coordinates show that the LTB4 cutoff value of 40.89 ng/mL used in this study has a sensitivity

Table 1 Characteristics of research subject

Demographic profile	Case *n* (%)	Control *n* (%)
Age median ± SD (years old)	56.5 ± 4.9	56.5 ± 5.2
Gender		
Male	16 (72.7)	14 (70.0)
Female	6 (27.3)	6 (30.0)
Duration of DM		
< 2 years	14 (63.6)	16 (80.0)
2–5 years	8 (36.4)	4 (20.0)
Type of diabetic drugs		
Oral	14 (63.6)	9 (45.0)
Insulin	8 (36.4)	11 (55.0)
LTB 4 serum		
Normal	5 (22.7)	12 (60.0)
High	17 (77.3)	8 (40.0)

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ROC Curve

Fig. 1 ROC result of serum LTB4 level and painful diabetic neuropathy with AUC 84.2%

Table 3 Bivariate analysis of other variables and painful diabetic neuropathy on type 2 DM patients

Variable	Case *n* (%)	Control *n* (%)	OR (CI 95%)	*p*
Anti-diabetic drug type				
Insulin	8 (42.1)	11 (57.9)	2.139 (0.62–7.37)	0.226
Oral	14 (60.9)	9 (39.1)		
DM duration				
< 2 years	14 (46.7)	16 (53.3)	2.286 (0.56–9.25)	0.315
2–5 years	8 (66.7)	4 (33.3)		

Table 4 Multivariate logistic regression analysis

Characteristics	Adjusted OR	CI 95%	*p*
Step 1			
High LTB4 level	10.431	1.056–102.997	0.045
Anti-diabetic drug type	0.394	0.041–3,782	0.420
Step 2			
High LTB4 level	5.10	1.336–19.470	0.017*

of 95.7% and a specificity of 80.3%. The research data were divided into two group; high serum LTB4 group at ≥ 40.89 pg/mL and normal serum LTB4 at < 40.89 pg/mL.

The analysis showed a significant correlation between high serum LTB4 levels with PDN in type 2 DM patients with OR 5.10 (1.34–19.4; $p = 0.014$) which means high serum LTB4 levels in type 2 DM patients increased risk 5.10 times to experience PDN compared with DM patients with low serum LTB4 levels. This can be shown in Table 2.

This study also sought a relationship between PDN in patients with type 2 diabetes with other variables that might be influential. In this study a bivariate analysis was carried out between diabetic neuropathy pain in patients with type of anti-diabetic drugs, and duration of anti-diabetic drugs in Table 3. The hypothesis test used was a paired categorical comparative test with the Chi-square method. Of all the variables, there were no statistically significant variables related to PDN in patients with DM.

To find out the variables that are independent risk factors for the occurrence of PDN in patients with DM, a multivariate analysis using logistic regression method

was performed. The variables included in the multivariate analysis were serum LTB4 levels and the type of oral anti diabetic drug which in the bivariate analysis obtained p values < 0.25. The complete data of multivariate analysis is presented in Table 4. From the multivariate analysis, it was found that the independent risk factor for the incidence of PDN in patients with DM was serum LTB4 levels with OR 5.10; 1.336–19.470; $p = 0.017$.

Discussion

This study found a significant relationship between high serum LTB4 levels and PDN in patients with DM. High serum LTB4 levels in DM patients significantly increased the risk of 5.10 times to suffer PDN compared with DM patients with low serum LTB4 levels (1.34–19.4; $p = 0.014$). Diabetes mellitus causes chronic low-grade inflammation and metabolic changes. This inflammation is triggered by various endogenous substances and causes sterile inflammation. This inflammation causes the release of arachidonic acid from leukocytes and arachidonic acid then metabolized into 5-HpETE (5-hydroperoxyeicosatetraenoic acid) and with the help of the 5-LO (5-lypoxygenase) enzyme becomes LTA4 (leukotriene

Table 2 Bivariate analysis of LTB4 serum level and painful diabetic neuropathy on type 2 DM patients

Variable		Case *n* (%)	Control *n* (%)	OR (CI 95%)	*p*
Leukotriene B4 Serum level	High	17 (77.3)	8 (40.0)	5.10 (1.34–19.4)	0.014*
	Normal	5 (22.7)	12 (60.0)		

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A4). LTA4 then metabolizes into LTB4 by LTA4 hydrolase and LTB4 can bind to its specific receptor [16].

LTB4 can also work centrally on the occurrence of PDN. LTB4 binds to the BLT1 receptor expressed in the dorsal medulla of the spinal cord. This activation causes an increase in the activation of NMDA (*N*-methyl-ᴅ-aspartate) receptors through intracellular G protein, besides that LTB4 can cause PDN by activating NADPH (Nicotinamide adenine dinucleotide phosphate) oxidase which causes neuronal damage [17, 18].

Study conducted by Noguchi and colleagues found that type 2 DM patients with high serum LTB4 levels have higher risk of suffering from painful diabetic neuropathic compared to the low serum LTB4 levels. This finding is thought to be due to increased leukotriene synthesis in the spinal cord after peripheral nerve injury and this enzyme affects pain behavior especially diabetic neuropathy pain. In addition, lipooxigenase metabolites are also involved in the process of hyperalgesia in peripheral inflammation. LTB4 is released by immune cells such as neutrophils and has a nociceptive effect on peripheral inflammation [19].

Expression of 5-LO in spinal cord microglia and BLT-1 mRNA in spinal cord neurons increases when peripheral nerve injury occurs. These findings indicate that central sensitization after peripheral nerve injury associated with the role of LTB4. However, it is not yet known how LTB4 plays a role in excitation of neurotransmitters in the spinal cord's dorsal horn [20]. According to author's knowledge, there has been no similar study comparing the levels of leukotrienes B4 serum with diabetic neuropathic pain in DM patients, but there are several studies linking levels of inflammatory mediators such as NFkB with the incidence of diabetic neuropathy in patients with DM [21].

Limitations in this study included a limited sample population, no electrophysiological examination was performed in all cases, and did not determine the minimum level of LBT4 that causes neuropathic pain.

Conclusion
From this study it was assumed that high serum LTB4 levels is a risk factor for painful diabetic neuropathy in Type 2 Diabetes Mellitus. The examination of serum LTB4 levels can be considered as a parameter to determine the risk of neuropathic pain in people with type 2 diabetes, so that early management can be done. However, further research with bigger sample size and usage of electrophysiology is needed.

Abbreviations
DM: Diabetes mellitus; PDN: Painful Diabetic Neuropathic; NF-kB: Nuclear factor-kappa B; LTB4: Leukotriene B4; BLT-1: Binds to leukotriene receptors-1; HIV: Human Immunodeficiency Virus; BDI II: Beck Depression Inventory II; 5-HpETE: 5-Hydroperoxyeicosatetraenoic acid; 5-LO: 5-Lypoxygenase; LTA4: Leukotriene A4; NMDA: *N*-Methyl-ᴅ-aspartate; NADPH: Nicotinamide adenine dinucleotide phosphate.

Declarations

Ethics approval and consent to participate
This research has obtained ethical clearance No:2969/UN14.2.2.VII.14/LP/2019 from Udayana University Faculty of Medicine/Sanglah General Hospital Research Ethics Committee and also has obtained written consent from patients to participate this study.

Consent for publication
This research has obtained consent for publication.

Competing interests
None (the authors declare that they have no competing interests).

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