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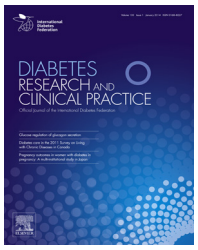

Quantitative evaluation of microvascular dysfunction in peripheral neuropathy with diabetes by indocyanine green angiography

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ABSTRACT

Aims: Peripheral neuropathy in diabetes (PND) plays a central role in foot ulceration with diabetes, and leads to an abnormal microvascular structure, including arteriovenous shunting. To assess the extent of arteriovenous shunting we performed indocyanine green angiography (ICGA) in patients with diabetes and evaluated quantitative ICGA parameters.
Methods: Between November 2012 and July 2013, twenty-six limbs in 14 patients with PND and twenty-three limbs in 15 patients without PND underwent ICGA testing. The ICGA parameters, which included the time to maximum intensity (Tmax), the time from fluorescence onset to half the maximum intensity (T1/2), the time elapsed from the maximum intensity to 90% of the Imax and to 75% of the Imax (Td 90% and Td 75%, respectively) and the rate of intensity measured 60 s after the Tmax to Imax (IR 60 s), were compared between the patients with and without PND.
Results: The Tmax, T1/2, Td 90%, Td 75% and IR 60 s were significantly different between patients with and without PND. A value of Td 90% >30 s was significantly correlated with the presence of PND (sensitivity: 0.85, specificity: 0.78).
Conclusions: ICGA tests can be used to quantitatively assess arteriovenous shunting in the limbs with PND. By measuring the value of the Td 90%, ICGA tests can estimate the presence of the arterio-venous shunting in PND, which might be helpful for assessing the progression of foot ulceration with diabetes, gangrene and the need for amputation.

1. Introduction

Foot ulcers with diabetes are a major healthcare problem, and foot complications are among the most serious and costly complications that occur in patients with diabetes. The risk of a person with diabetes undergoing a lower extremity amputation is estimated to be 23 times that of a person without diabetes [1]. Peripheral neuropathy in diabetes (PND), ischemia and infection are the principal pathogenic factors that cause foot disease associated with diabetes. PND has a central role in the development of diabetic foot ulcers, and is present in over 80% of patients with foot lesions with diabetes [2]. In the course of studying many aspects of PND, a common observation has been that the foot was warm and often had an easily palpable and visible pulse at the dorsal pedis artery. Arteriograms have shown increased vascularity with rapid flow through the dilated vessels of the feet and early filling of

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the venous circulation [3]. These findings suggest that there might be abnormal arteriovenous shunting, and led to the identification of several functional abnormalities of the microvasculature, including an increase in arteriovenous shunting and impaired vasoreactivity [4]. Therefore, foot ulceration with diabetes and arteriovenous shunting have been linked to PND [5], and the identification of impaired microvascular blood flow and vasoreactivity may lead to the early identification of patients at risk for peripheral arterial disease. However, few studies have reported the quantitative evaluation of the microvascular blood flow due to abnormal arteriovenous shunting [3,6].

Indocyanine green (ICG) has been clinically used as a near infrared fluorophore for intravital imaging, as a marker of liver function [7] and as a sensitizer for photodynamic therapy [8]. After intravenous administration, ICG is distributed throughout the intravascular space, where it is rapidly bound to the major serum proteins, especially albumin [9]. ICG angiography (ICGA) with ICG injection can rapidly provide excellent and informative images of tissue perfusion, and allows for the estimation of the tissue perfusion [10].

The aim of this study was to evaluate quantitative information of peripheral perfusion in limbs with diabetes. We also investigated the degree of arteriovenous shunting by ICGA tests, which might estimate the presence of PND in limbs with diabetes.

2. Materials and methods

2.1. Patients

In this study, we included ICGA tests, which were performed in twenty-nine patients with diabetes, who were admitted and underwent further investigations and/or revascularization procedures for the symptoms of peripheral arterial disease (PAD) at Tokyo Medical and Dental University Hospital between November 2012 and July 2013. All patients had type 2 diabetes. We excluded patients with conditions associated with abnormal arteriovenous shunting such as bypass procedures, critical limb ischemia, and venous insufficiency, including deep venous thrombosis and/or varicose veins.

The presence of PND was investigated by clinical signs, including paresthesias, dysesthesias and the loss of sensation and/or by a neurophysiological test of the perception of vibration using a tuning fork. The tuning fork was applied on a bony part on the dorsal side of the distal phalanx of the first toe. The test was considered to be positive (the presence of PND) if the patient met two of three criteria [11]. Twenty-six limbs in 14 patients with PND and twenty-three limbs in 15 patients without PND underwent ICGA testing in this study. All protocols, surveys and consent forms were approved by the Institutional Review Board of Tokyo Medical and Dental University Hospital. Written informed consent was obtained from all subjects.

All limbs were divided in two groups, those with PND and those without PND. The patient and limb characteristics are described below. Hypertension was diagnosed as a systolic blood pressure $>$ 140 mmHg or a diastolic blood pressure $>$ 80 mmHg or having been treated for hypertension. Dyslipidemia was defined as a serum LDL cholesterol $>$ 140 mg/dl (3.6 mmol/l) or HDL cholesterol $<$ l40 mg/dl (1.03 mmol/l) or triglycerides $>$ 150 mg/dl (1.7 mmol/l), or having been treated for dyslipidemia. Coronary artery disease was defined as the presence of angina pectoris or myocardial infarction, or both, and was documented by coronary angiography or a history of any revascularization of the coronary arteries. Cerebrovascular disease was defined as a history of stroke, transient ischemic attacks, carotid artery revascularization or cerebral hemorrhage. The ankle-brachial pressure index (ABI), toe-brachial pressure index (TBI) and toe pressure (TP) were measured to assess the severity of the ischemia. We used the VasoGuard P84^{TM} system (SciMed Ltd., Bristol, UK) to measure the ABI, TBI and TP on the same day the ICGA test was performed.

2.2. ICGA testing

The tested patient was in supine position and measurements were performed at a room temperature of 20–25 °C. A 0.1 mg/kg dose of ICG (Diagnogreen^{TM}; Daiichi-Sankyo Pharmaceutical, Tokyo, Japan) was intravenously injected via a peripheral venous line. Immediately after the injection of ICG, fluorescence images were obtained using an infrared camera system (Photodynamic Eye^{TM}, Hamamatsu Photonics K.K., Hamamatsu, Japan), which activated the ICG with emitted light at a wavelength of 760 nm and filtered out light with a wavelength of $<$820 nm. The light source for the emission of ICG consisted of 760 nm light-emitting diodes, and the detector was a charge-coupled device camera. The camera system was positioned over the foot at 20 cm from the skin. After the injection of ICG, we captured and recorded the image for 5 min. Real-time fluorescence images were displayed on a monitor and recorded using the digital image processing method provided by the Audio Video Interweave system, and the time–intensity curve was calculated (Fig. 1a–f). ICG is nontoxic, and has a very low incidence of adverse reactions (1/40,000 doses) [12]. In all cases, the hemodynamic parameters, such as the pulse rate and blood pressure, were within the normal ranges.

2.3. ICGA image analysis

For the comparative measurements, we set the region of interest as whole dorsum foot from the Chopart joint to the distal part of the metatarsal bones. In order to evaluate the ICGA tests quantitatively, multiple parameters were obtained and analyzed to assess the peripheral perfusion. These parameters included the time from ICG onset to maximum intensity (Tmax), the magnitude of intensity from ICG onset to maximum intensity (Imax), the time elapsed from the fluorescence onset to the half value of the maximum intensity (T1/2), the time elapsed from the maximum intensity to 90% of the Imax and to 75% of the Imax (Td 90% and Td 75%, respectively) and the rate of intensity measured 60 s after the Tmax to Imax (IR 60 s) (Fig. 2). The image processing and data analysis were performed using the ROIs^{TM} version of the U11437 software program (Hamamatsu Photonics K.K., Hamamatsu, Japan).

2.4. Statistical analysis

The continuous variables are expressed as the means ± SD (standard deviation), and categorical variables are expressed