Diabetic Neuropathy: Function-Structure of Corneal Nerves to Assess Injury - Repair

Running title: corneal sensitivity and diabetic neuropathy

The study is catalogued NCT04222660.

Study Protocol

The purpose of this study is to test a new approach for the early detection of DPN. Diabetes patients meeting the criteria were recruited from the Diabetes Clinics and non-diabetic subjects from the general veteran population. A written and informed consent was obtained from all participants. The study adhered to the Declaration of Helsinki and was approved by the Institutional Review Board (#201610799). Participants were divided into two groups: diabetic with peripheral neuropathy and non-diabetic with no history of peripheral neuropathy. Inclusion Criteria: 1) Type 2 diabetes diagnosed based on the opinion of an Endocrinologist, the absence of a history of ketoacidosis, and a C-peptide > 0.8 ng/ml. 2) At least 10 year known duration of diabetes. 3) Age ≥ 50 yr. 4) HbA1c < 9.0%. 5) Mild to moderate PN based on response to Michigan Neuropathy Screening Instrument (MNSI),[18] nerve conduction and amplitude determined using DPNCheck, medical record, presence or absence of ankle reflex, vibration threshold and 10g monofilament sensation. Exclusion Criteria: 1) History of neurologic, muscular, genetic, or other condition known to affect muscle function or exercise tolerance. 2) Electrolyte abnormalities, untreated hypothyroidism, abnormalities in calcium, phosphate, or magnesium concentrations, or any other metabolic disturbance affecting neural function. 3) Cigarette smoking in the past year. 4) Peripheral vascular, cardiac, pulmonary, or any other disorder affecting blood or tissue oxygenation. 5) Use of any medication known to alter peripheral nerve function. 6) History of diabetic foot ulcer. 7) History of corneal disease including dry eye. 8) History of wearing contacts. 9) Any other medical or psychological condition judged to limit compliance with the protocol or interpretation of results.

The study included 28 patients with type 2 diabetes and peripheral neuropathy (24 male and 4 female). The average age of the diabetes patients was 66.8 ± 1.9, with duration of diabetes 20.0 ± 2.6 and hemoglobin A_{1C} 8.2 ± 0.3. The non-diabetic subjects (11 male and 5 female) were younger 49.8 ± 5.0.

Clinical neuropathy assessment: The MNSI questionnaire was self-administered. Each applicant was encouraged to answer yes or no to each of the 15 questions. A score of ≥ 4 was considered abnormal. During the MNSI examination a health professional examined the feet, for deformities, dry skin, calluses, infections, and ulcers. Quantitative sensory testing included the presence or absence of ankle reflexes and vibration and monofilament sensation at the dorsum of the great toe.[19] Nerve conduction values were determined using a point of care device *DPN*Check (*DPN*Check, NeuroMetrix, Inc., Waltham, MA). Manufacture instructions were followed, briefly after skin preparation, the probes were coated with the conductive gel and applied directly to the skin, posterior to the lateral malleolus.[20] Up to four recordings were made as individual tolerance allowed. The recordings were averaged.

Ophthalmic assessment: All patients underwent a visual acuity assessment. Provided answers to two questionnaires the Ocular Surface Disease Index (OSDI) and Dry Eye Questionnaire (DEQ-5). To evaluate cornea sensitivity each patient underwent a Cochet-Bonnet aesthesiometer examination and blinking response determination following application of a drop of isotonic saline (308 mosmol/liter) or Muro-128 solution 5% sodium chloride (1710 mosmol/liter) (Rugby Laboratories, Livonia, MI). This study was performed by having the subject rest their chin comfortably on a support. Three CMOS cameras (Imaging Development Systems GmbH, Obersulm, Germany), one positioned in front of the subject and two positioned laterally, were used to observe both eyes simultaneously. Custom software was used to synchronize video streams and obtain images (MATLAB R2012a, The MathWorks Inc., Natick, MA). Video was taken continuously at 30fps of each subject as an isotonic solution was delivered to the left eye. The response was video recorded for 60 sec after a 10 sec delay. After a 15 min wash and rest period the procedure was repeated using a drop of Muro-128 solution 5% sodium chloride. An image collector was used offline to retrieve video frames every 10 seconds from each eye drop concentration epoch; therefore 15 images were analyzed subject per solution. Fiji image analysis software was used by a masked technician to measure

the visible surface area of both eyes between the upper and lower eyelids. In each recording epoch, the ratio of time the eyelids were closed are presented as a percentage of the subjects' maximum visible eye surface area between the eyelids during that epoch.

Data Analysis

Results are presented as mean ± S.E.M. Comparisons between the control and diabetic subjects were conducted using Student t-test (Prism software; GraphPad, San Diego, CA). A p value of less than 0.05 was considered significant.