CENTRAL AND PERIPHERAL NEURODEGENERATION IN DIABETES AND DEMENTIA

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CENTRAL AND PERIPHERAL NEURODEGENERATION IN DIABETES AND DEMENTIA

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Preface

I have been involved in clinical research in the field of diagnostic biomarkers of diabetic neuropathy since I joined Prof. Rayaz Malik's team in January 2010 at the University of Manchester and subsequently as a clinical researcher in Weill Cornell Medicine in Qatar (WCM-Q). I have published (50) papers, 18% of which I am the lead author. According to Google Scholar, as of July 2020, I have a h-index of 20 and i10-index of 29. I have established the study '*prevalence and risk factors of diabetic neuropathy and painful diabetic neuropathy in Qatar'*. I have studied the effect of a GLP-1 agonist and insulin sensitizer compared to insulin on corneal nerve regeneration in poorly controlled T2DM patients in a clinical trial. I have assessed the diagnostic ability of corneal confocal microscopy (CCM) as a biomarker for neurodegeneration in dementia and schizophrenia. I have been involved in the writing of successful grant proposal: for the Dementia Project (NPRP12S-0213-190080) awarded $695,903 by Qatar Foundation, the DELPHIC-Qatar (delirium) project (IRGC-04-SI-17-153) awarded $234,000, and the Schizophrenia study (IRGC-04-SI-17-166) awarded $122,000 by Hamad Medical Corporation.

Abstract

Background: Diabetic peripheral neuropathy (DPN) affects ~50% of people with diabetes and leads to painful DPN (pDPN), diabetic foot ulceration (DFU) and amputation imposing a significant health and economic burden. Given that there are currently no European Medicines Agency (EMA) and FDA approved therapies for DPN it is important to establish the current prevalence and modifiable risk factors for DPN and assess the benefit of treatments utilizing corneal confocal microscopy (CCM), a sensitive technique to quantify early nerve regeneration in DPN. Furthermore, CCM has shown corneal nerve loss in central neurodegenerative disorders such as Parkinson's disease and multiple sclerosis, therefore the diagnostic utility of this technique was assessed in subjects with mild cognitive impairment (MCI) and dementia.

Aims: This work established the prevalence and risk factors of DPN and those at high risk of DFU in **Chapter 3;** the prevalence and risk factors of pDPN in **Chapter 4** and the prevalence of DPN and pDPN in both primary (PHC) and secondary health care (PHC) in type 2 diabetes (T2D) in Qatar in **Chapter 5**. It investigated the effect of hypertension on neuropathic symptoms and deficits in type 1 diabetes (T1D) in **Chapter 6**, the association between metformin induced B_{12} deficiency and DPN in **Chapter 7**, and the effect of exenatide and pioglitazone or basal-bolus insulin on DPN in patients with poorly controlled T2D as an exploratory sub-study of the Qatar study, an open-label, randomized controlled trial (clinicaltrials.gov identifier NCT02887625) in **Chapter 8**. It assessed the association of corneal nerve morphology with cognitive impairment in MCI and dementia in **Chapter 9** and compared the diagnostic ability of CCM to visual rating of medial temporal lobe atrophy (MTA) on brain MRI to distinguish subjects with MCI or dementia from subjects with no cognitive impairment (NCI) in **Chapter 10**.

Methods: All the research work was conducted in Qatar apart from Chapter 6 which was performed in Manchester, UK. The study design, inclusion and exclusion criteria, diagnosis and assessments for each study are described in detail in the methods section in each chapter. Subjects were randomly enrolled and screened for eligibility on the day they attended the

clinic. Demographic, clinical and metabolic characteristics and list of medications were recorded. Subjects underwent assessment of DPN and pDPN (Chapter 3-7), CCM (Chapter 6, 8-10), intraepidermal nerve fiber density (Chapter 6), autonomic neuropathy (Chapter 6), quantitative sensory testing (QST) for warm and cold perception (Chapter 6), nerve conduction studies (NCS) (Chapter 6), diagnosis of NCI, MCI and dementia (Chapter 9 & 10), cognitive screening (Chapter 9 & 10), functional independence screening (Chapter 9 & 10), brain MRI and MTA visual rating (Chapter 10).

Results: Chapter 3 established that the prevalence of DPN and high risk of DFU in those with DPN were 23.0% and 33.7%, respectively in SHC (n=1,095) and the risk factors were age, duration of diabetes, poor glycemic control, hyperlipidemia and hypertension. **Chapter 4** established that the prevalence of pDPN was 34.5% in SHC and the risk factors were DPN, obesity, physical activity and smoking. **Chapter 5** shows that PHC (n=298) had a significantly lower prevalence of DPN (14.8%, P=0.001) and pDPN (18.1%, P<0.0001) but comparable high risk for DFU (31.8%, P=0.3) compared to SHC. Alarmingly, 79.5-82.3% of patients with DPN were undiagnosed in PHC and SHC. **Chapter 6** shows that hypertension affects NCS in patients with T1D after controlling for HbA1c, cholesterol, triglycerides, and BMI but has no impact in subjects without diabetes. **Chapter 7** shows no difference in DPN or pDPN between those with and without B_{12} deficiency and between metformin and non-metformin users. **Chapter 8** shows that a combination of exenatide once weekly and pioglitazone or basal-bolus insulin leads to corneal nerve regeneration detected by CCM, but no change in neuropathic symptoms or sudomotor function. **Chapter 9** shows that CCM identified corneal nerve loss and associated it with cognitive and functional decline in MCI and dementia. **Chapter 10** shows that CCM had comparable diagnostic ability for dementia with MTA whilst only CCM can distinguish MCI from NCI, after adjustment for diabetes.

Conclusions: Despite DPN affecting 23% of adults with T2D in SHC and 15% in PHC, ~80% of patients with DPN were undiagnosed in both PHC and SHC, highlighting the need for implementing annual DPN screening. The lower prevalence of DPN and pDPN in PHC compared to SHC may be attributed to better overall risk factor control in PHC and referral bias as patients who are poorly managed with complications are referred to SHC. The identification of hyperglycemia, hyperlipidemia and hypertension as modifiable risk factors

for DPN and obesity and physical activity as modifiable risk factors of pDPN provide potential treatments for the management of DPN and pDPN. The association between hypertension and NCS further supports the role of hypertension in DPN. Our study does not confirm that DPN was associated with B_{12} levels and metformin use in Qatar. Treatment with exenatide and pioglitazone or basal-bolus insulin resulted in corneal nerve regeneration, but no change in neuropathic symptoms or sudomotor (control of sweat glands activity) function over 1 year, highlighting the importance of selecting appropriate endpoints to show treatment efficacy in DPN. CCM had a better diagnostic outcome for identifying subjects with MCI and comparable with dementia compared to MTA rating and should be considered as an objective imaging marker of neurodegeneration in MCI and dementia.

Contents

List of figures

Figure 2.1. PhD project timeframe. 20
Figure 2.2. Flow chart showing the management of this PhD. 21
Figure 2.3. Neurothesiometer devices used to assess for impaired vibration perception threshold. Georgios Ponirakis (PhD student) in the photo. 26
Figure 2.4. The Heidelberg Retinal Tomograph III device coupled with the Rostock Cornea Module used for corneal confocal microscopy (CCM). Prof. Rayaz Malik (PhD mentor) on the far right and Georgios Ponirakis (PhD student) performing CCM analysis in the photo.....27
Figure 2.5. MRI brain performed on a superconductive magnet operated at 3T (Skyra, Siemens) at the MRI unit in Rumailah Hospital. 31

List of tables

Abbreviations:

Alzheimer's disease (AD)
Cardiac autonomic neuropathy (CAN)
Cold perception threshold (CPT)
Corneal confocal microscopy (CCM)
Corneal nerve branch density (CNBD)
Corneal nerve fiber density (CNFD)
Corneal nerve fiber length (CNFL)
Diabetic autonomic neuropathy (DAN)
Diabetic foot ulceration (DFU)
Diabetic peripheral neuropathy (DPN)
Diastolic blood pressure (DBP)
Hypertensive (HT)
Intra epidermal nerve fiber density (IENFD)
Medial temporal lobe atrophy (MTA)
Mild cognitive impairment (MCI)
No cognitive impairment (NCI)
Neuropathy Disability Score (NDS)
Normotensive (NT)
Painful diabetic peripheral neuropathy (pDPN)
Peroneal compound motor action potential (PCMAP)
Peroneal motor nerve conduction velocity (PMNCV)
Sural nerve conduction velocity (SNCV)
Sural nerve action potential (SNAP)
Systolic blood pressure (SBP)
Tibial compound motor action potential (TCMAP)
Tibial motor nerve conduction velocity (TMNCV)
Type 1 diabetes (T1D)
Type 2 diabetes (T2D)
Vibration perception threshold (VPT)
Warm perception threshold (WPT)

Chapter 1: General introduction

1.1 Diabetic peripheral neuropathy

Diabetic peripheral neuropathy (DPN) is a common complication of diabetes, affecting ~50% of people with diabetes (Tesfaye and Selvarajah, 2012). It is a progressive neurodegenerative disorder of the peripheral nervous system involving sensory, autonomic and motor nerve fibers. The clinical diagnosis of DPN is challenging due to the insidious onset of disease and gradual decline of peripheral nerve function (Malik, 2020). Damage to the peripheral nerve fibers occurs in a distal symmetrical manner. It imposes a significant health and economic burden to both the patient and health care providers (Raghav et al., 2018). DPN leads to painful DPN (pDPN) in 18-65% (Ponirakis et al., 2019b), erectile dysfunction in 53-73% (Kouidrat et al., 2017) and diabetic foot ulceration (DFU) in 2-17% (Raghav et al., 2018) of patients with type 2 diabetes (T2D). Painful DPN has a significant impact on the patient's quality of life (Girach et al., 2019) as it is accompanied by depression, anxiety and sleep disturbance. The prevalence of DPN increases with age and duration of diabetes (Young et al., 1993, Cabezas-Cerrato, 1998).

1.1.1 Prevention

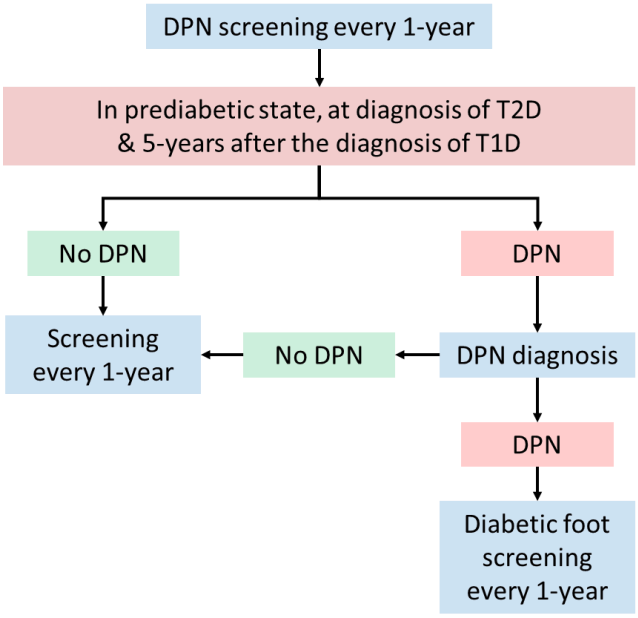
The management of DPN and its consequences has primarily focused on: **1.** Screening to identify early DPN; **2.** Management of risk factors to prevent or delay DPN; **3.** Screening for those at risk of DFU to prevent ulceration and amputation.

Screening annually for symptoms and signs of DPN starting at diagnosis of T2D and 5-years after the diagnosis of type 1 diabetes (T1D) is recommended by the 2017 American Diabetes Association (ADA) position statement on DPN (Pop-Busui et al., 2017) (Figure 1.1). Screening for DPN in prediabetes is also advocated based on the higher prevalence of impaired glucose tolerance and metabolic syndrome in people presenting with painful neuropathy. DPN (Boulton and Malik, 2010) and small nerve fiber damage (Azmi et al., 2015) have been reported in patients with impaired glucose tolerance (IGT). However, despite having a serious impact on the patient's quality of life and outcomes, screening for DPN and pDPN remains

inadequate. An alarmingly high prevalence of undiagnosed DPN 51-82% (Wang et al., 2011, Herman and Kennedy, 2005) and pDPN 13-62% (Ziegler et al., 2018, Daousi et al., 2004) have been reported. The diagnosis of DPN is often made during diabetic foot screening or after the occurrence of diabetic foot ulceration in which case DPN has not been diagnosed for some time. Patients with painful symptoms are often unaware that the pain is related to diabetes and do not report it to their clinician (Daousi et al., 2004, Eichholz et al., 2017).

The key to prevention of DPN is the identification and optimal management of risk factors (Pop-Busui et al., 2017), given the lack of disease modifying treatments for DPN (Malik, 2016, Malik, 2014). Optimization of glycemic control may prevent DPN onset and delay DPN progression (Pop-Busui et al., 2017). In the DCCT, intensive insulin treatment reduced the incidence of clinical DPN by 60% (Diabetes et al., 1993) and prevented peroneal nerve conduction velocity slowing over a 5-year period in patients with T1D. The Kumamoto study (Ohkubo et al., 1995) showed that intensive treatment prevented nerve conduction slowing over 6 years and the ACCORD trial (Ismail-Beigi et al., 2010) showed a reduction in the incidence of loss of ankle reflexes but no effect on VPT over 6-years (Callaghan et al., 2012). However, in patients with T2D the UKPDS (UK Prospective Diabetes Study (UKPDS) Group, 1998) and VA-CSDM trial (Azad et al., 1999) reported that intensive treatment had no effect on the incidence of DPN and CAN compared with conventional treatment, suggesting possible other important factors. These involve cardiovascular risk factors including hypertension (Kesavamoorthy et al., 2015, Yang et al., 2015) and hyperlipidemia (Tesfaye et al., 2005, Smith and Singleton, 2013), the management of which may also prevent DPN; angiotensin converting enzyme (ACE) inhibitors (Malik et al., 1998, Ruggenenti et al., 2011, Reja et al., 1995) and statins (Davis et al., 2008, Villegas-Rivera et al., 2015) have both shown to prevent or slow the progression of DPN. Lifestyle interventions are also important, including physical activity (Al-Kaabi et al., 2014, Smith et al., 2006) and avoidance of smoking (Al-Mahroos and Al-Roomi, 2007, Tesfaye et al., 2005). Weight loss may improve symptoms of pDPN (Jambart et al., 2011, Van Acker et al., 2009, Ziegler et al., 2018) and a study by Smith et al. reported that lifestyle intervention in patients with pre-diabetes reduced neuropathic symptoms and improved small fiber function and structure (Smith et al., 2006).

Figure 1.1. Proposed screening protocol for DPN in clinical practice.



Screening annually for DPN starting at diagnosis of T2D and 5-years after the diagnosis of T1D is recommended by the American Diabetes Association (ADA). If the screening result shows DPN, further assessments are required to confirm diagnosis of DPN. After further assessments of DPN if the symptoms and signs do not meet the criteria for diagnosis of DPN, further annual screening for DPN is recommended. However if the symptoms and signs meet the criteria for diagnosis of DPN, annual screening for diabetic foot is recommended. Abbreviation: Diabetic peripheral neuropathy (DPN), type 1 diabetes (T1D), type 2 diabetes (T2D).

One in four patients with DFU are at risk of amputation (Apelqvist and Agardh, 1992). Screening annually for patients at risk of DFU is advocated by the International Working Group on the Diabetic Foot (IWGDF) include regular examinations for DFU, educating the patient and family about appropriate foot care, routine wearing of appropriate footwear and treating risk factors for DFU (Bus et al., 2020). The National Diabetes Foot Care Audit (NDFA) in the UK showed that patients who referred themselves directly to a foot care clinic had higher healing rates after 12 weeks compared to those referred by a health professional (56% vs 32-48%) (Mayor. S, 2017). Despite the 5-year mortality of people with a DFU being higher than many common cancers (Moulik et al., 2003, Armstrong et al., 2007), the development of DFU in patients who have not been screened remains alarmingly high (Wang et al., 2011, Herman and Kennedy, 2005).

1.1.2 Screening and diagnosis

The purpose of screening for DPN is to use a single rapid test routinely for all patients with diabetes in a busy clinic and identify patients with DPN (Figure 1.1). Screening tests for DPN should be able to detect incipient nerve damage before the development of overt clinical diabetic neuropathy especially in pre-diabetes or early diabetes. Potential screening methods for DPN include: Sudomotor function (Sudoscan (2 minutes) (Selvarajah et al., 2015) or Neuropad test (10 minutes) (Ponirakis et al., 2014), quantitative sensory tests (QST) including vibration perception threshold (VPT) (3 minutes) (Bril and Perkins, 2002a) or NerveCheck for testing vibration, cold, warm perception and hyperalgesia to thermal induced pain (10 minutes) (Ponirakis et al., 2016); composite scoring systems that include symptoms, signs, or both to quantify neuropathic deficits including the Neuropathy Disability Score (NDS) (5 minutes) (Young et al., 1993), modified Toronto Clinical Neuropathy Score (mTCNS) (5-10 minutes) (Bril and Perkins, 2002b) and Michigan Neuropathy Screening Instrument (MNSI) (10 minutes) (Feldman et al., 1994).. However, the most commonly advocated test is the evaluation of pressure sensation using the 10g-monofilament which assesses severe neuropathy and those at risk of diabetic foot ulceration (Pop-Busui et al., 2017), but continues to be recommended for DPN screening (Perkins et al., 2010). Moreover, the validity of this method as a screening test for DPN is further challenged given that a study has shown that it failed to detect DPN in ~80% of patients with DPN regardless of the number of sites 3, 4 or 10 tested on each foot (Zhang et al., 2018).

The diagnosis of DPN is based on a comprehensive assessment of history, symptoms and signs to rule out other causes of peripheral neuropathy including autoimmune diseases (Sjogren's syndrome, lupus, rheumatoid arthritis), infections (HIV, hepatitis B and C), inherited (Charcot-Marie-Tooth), inflammatory (CIDP), tumors, vitamin B_{12} deficiency, hypothyroidism, alcoholism and injury or pressure on the nerve. Symptoms of DPN include burning pain, numbness, tingling, pins and needles like pain, electric shocks, cold pain, allodynia (pain due to a stimulus that does not usually provoke pain) and hyperalgesia (increased pain from a stimulus that usually provokes pain). Early DPN is caused predominantly by small fiber neuropathy, especially in pre-diabetes (Azmi et al., 2015, Divisova et al., 2012) with involvement of large fibers as the disease progresses. Assessments for small fiber neuropathy

(Azmi et al., 2019d) include QST for warm and cold perception threshold (Bril and Perkins, 2002a, Ponirakis et al., 2016), pDPN assessment (Spallone et al., 2012), diabetic autonomic neuropathy (DAN) (Spallone et al., 2011), corneal nerve fiber morphology using corneal confocal microscopy (CCM) (Petropoulos et al., 2013c, Petropoulos et al., 2014, Petropoulos et al., 2013a, Malik et al., 2003, Ahmed et al., 2012) and intra-epidermal nerve fiber density (IENFD) from skin biopsy (Lauria et al., 2010a, Lauria et al., 2010b). Assessments for large fiber neuropathy (Azmi et al., 2019d) include VPT testing and nerve conduction studies (NCS)/electrophysiological studies (Kahn, 1992, Bril et al., 1998). DAN, results from the impairment of the sympathetic and parasympathetic nervous system and affects the cardiovascular, gastrointestinal, genitourinary and sudomotor systems (Spallone et al., 2011). Cardiovascular autonomic neuropathy (CAN) is the most studied and clinically important form of DAN. CAN is assessed by a battery of autonomic function tests including deep breathing heart rate variability (DB-HRV), Valsalva maneuver or postural blood pressure change (Olney, 1998). CAN is associated with an increased risk of silent myocardial ischemia and mortality (Vinik et al., 2003). Other techniques for testing DAN include sudomotor function testing and laser doppler imager flare response. Composite scoring systems that quantify neuropathic deficits of both small and large fiber neuropathy include the NDS (Young et al., 1993), mTCNS (Bril and Perkins, 2002b), Neuropathy Impairment Score of the lower limb (NIS-LL) (Bril, 1999), Total Neuropathy Score-clinical (TNS-C) (Cornblath et al., 1999), Michigan Diabetic Neuropathy Score coupled with the MNSI (Feldman et al., 1994) and Utah Early Neuropathy Score (UENS) (Singleton et al., 2008).

The screening and diagnosis of pDPN is based on a subjective description of specific painful symptoms experienced in the distal parts of the limbs and expressed in relation to intensity and frequency by the patient. Screening for pDPN should distinguish neuropathic pain from nociceptive or other types of chronic pain. Screening methods for pDPN include the Douleur Neuropathique 4 (DN4) questionnaire (Spallone et al., 2012), the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) pain scale (Bennett, 2001), the Neuropathic Pain Scale (NPS) (Jensen et al., 2006), the Neuropathic Pain Symptom Inventory (NPSI) (Bouhassira et al., 2004), and the Diabetic Peripheral Neuropathic Pain Impact measure (DPNPI) (Brod et al., 2015). The diagnosis of pDPN is based on the presence of painful symptoms and small fiber neuropathy.

The purpose of diabetic foot screening is to identify patients with advanced DPN who are at high risk of DFU and amputation. Those at risk of diabetic foot ulceration can be screened by testing pressure perception using the 10-g monofilament or Ipswich Touch Test by touching the tips of the toes of both feet with the index finger (Sharma et al., 2014), testing vibration perception using the 128-Hz tuning fork or Neurothesiometer to assess for VPT >25 Volts and absence of ankle reflexes (Boulton et al., 2008).

1.1.3 Management

There are currently no European Medicines Agency (EMA) and FDA approved disease modifying treatments for DPN (Malik, 2016, Malik, 2014) and only three approved medications for painful neuropathy, including duloxetine, pregabalin and tapentadol (Javed et al., 2015). Treatment with angiotensin converting enzyme (ACE) inhibitor (Malik et al., 1998, Ruggenenti et al., 2011, Reja et al., 1995) may improve neuropathy and statins (Arya et al., 2018, Hsu et al., 2017) and fibrates (Rajamani et al., 2009) may reduce amputation. Whilst optimization of glycemic control may prevent DPN onset and delay DPN progression in type 1 diabetes (T1D) (Pop-Busui et al., 2017), there are conflicting data on the benefits of improved glycemic control on DPN in T2D (Ohkubo et al., 1995, Ismail-Beigi et al., 2010, Pop-Busui et al., 2013, Azad et al., 1999, Gaede et al., 2003). Modification of cardiovascular risk factors including hypertension (Kesavamoorthy et al., 2015, Yang et al., 2015) and hyperlipidemia (Tesfaye et al., 2005, Smith and Singleton, 2013) may prevent DPN onset and delay DPN progression. Lifestyle interventions, including physical activity (Al-Kaabi et al., 2014, Smith et al., 2006), avoidance of smoking (Al-Mahroos and Al-Roomi, 2007, Tesfaye et al., 2005) and weight loss (Jambart et al., 2011, Van Acker et al., 2009, Ziegler et al., 2018) may reduce the incidence of DPN.

1.1.4 The contribution of this PhD thesis to the current knowledge on DPN prevention and management

According to the International Diabetes Federation, the prevalence of diabetes in adults aged 20-79 years in Qatar was 15.5% in 2020 (IDF Middle East and North Africa Region, 2020,), which is almost two-fold greater than the 2019 reported prevalence of 8.3% in the rest of the world (International Diabetes Federation, 2019,). The high prevalence of diabetes can be

translated into an increase in the prevalence of DPN. Indeed, in Qatar, 25% of patients attending secondary care were being seen for foot problems (Al-Thani et al., 2019). In 2015, Qatar launched the National Diabetes Strategy to improve the management of people with diabetes and its complications by establishing common clinical care pathways within and between primary and secondary health care.

The prevalence and risk factors of DPN have not been systematically studied in Qatar. Whilst the prevalence and risk factors of DPN from other countries is relevant, identifying the prevalence and risk factors of DPN in Qatar is key to planning the National Diabetes Strategy on preventing the complications of diabetes. **Chapter 3** has established the prevalence and risk factors in patients with T2D for DPN and those at high risk of DFU in secondary health care. **Chapter 4** has established the prevalence and risk factors of pDPN in patients with T2D in secondary health care. **Chapter 5** has compared the prevalence and risk factors of DPN and pDPN between primary and secondary health care.

Clinical and experimental studies suggest that hypertension is an independent risk factor for DPN in patients with T1D (Tesfaye et al., 2005, Forrest et al., 1997, Cavusoglu et al., 2015, Elliott et al., 2009, Sanada et al., 2015, Gregory et al., 2012) and T2D (Cardoso et al., 2015, De Visser et al., 2014, Kesavamoorthy et al., 2015, Yang et al., 2015). ACE inhibitors have been shown to improve NCS but there are conflicting data on the effect on neuropathic symptoms and other neuropathy measures (Malik et al., 1998, Ruggenenti et al., 2011, Reja et al., 1995). **Chapter 6** has identified the impact of hypertension on both large and small fiber measures in patients with and without T1D.

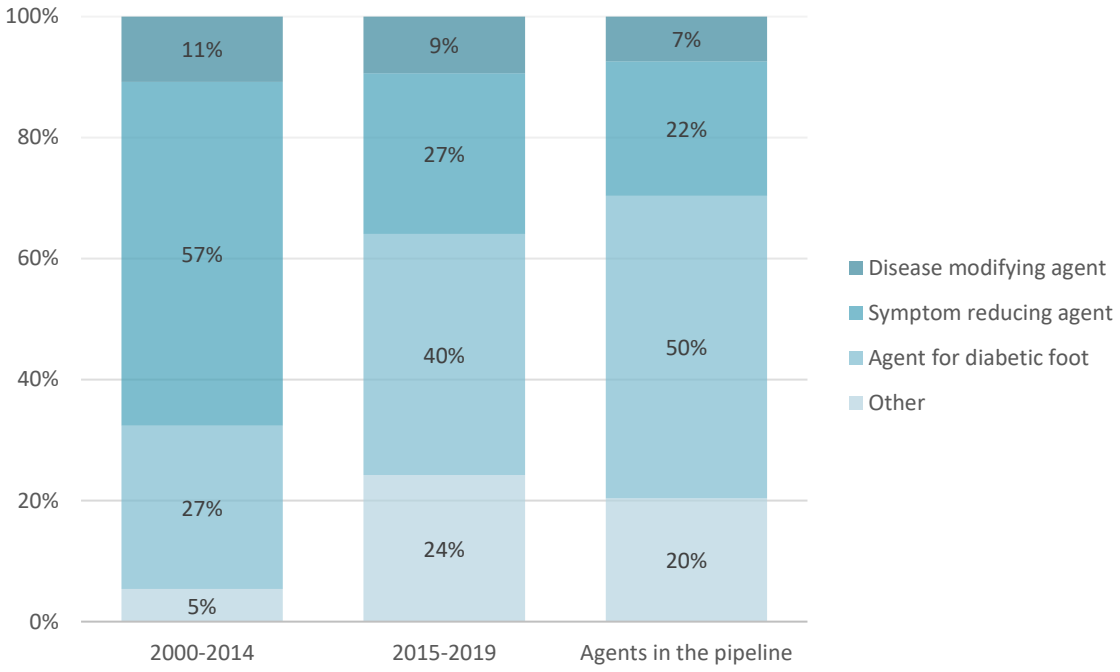
Most international guidelines recommend metformin after lifestyle intervention for T2D patients. This rationale is based on its 40-year long-term safety record and the fact that it has shown a 31% reduced incidence of T2D and 17% reduced incidence of metabolic syndrome at 2.8-years (Knowler et al., 2002). Despite conflicting data regarding the effect of metformin therapy on B_{12} deficiency (Chapman et al., 2016), a number of observational and placebo-controlled studies have confirmed that metformin may reduce vitamin B_{12} levels. A potential consequence of B_{12} deficiency is that it could result in or exacerbate DPN. However, there are conflicting reports on the association between metformin induced B_{12} deficiency and neuropathy, with some reports showing an association (Singh et al., 2013, Roy et al., 2016)

whilst others have refuted this (Khan et al., 2017, Russo et al., 2016, Ahmed et al., 2016, Ma et al., 2015). **Chapter 7** has determined whether treatment with metformin is associated with B_{12} deficiency and whether B_{12} deficiency is associated with DPN and painful diabetic neuropathy.

1.2 Development of disease-modifying agents for diabetic peripheral neuropathy

In total, 423 interventions have been evaluated for DPN in clinical trials between February 1998 to June 2020 (clinicaltrials.gov). There were 42 (9.9%) disease modifying agents, 183 (43.3%) agents for neuropathic pain, 143 (33.8%) agents for diabetic foot and 55 (13.0%) interventions such as diet, lifestyle, wound dressings, education, procedures and devices. Of concern, all trials of disease modifying agents have failed and the focus of interventions has shifted to agents for management of diabetic foot disease (Malik, 2016). Figure 1.2 shows that the proportion of disease modifying agents and symptom-reducing agents have been reduced by half, whilst the proportion of agents for diabetic foot have doubled and alternative non-drug interventions have increased 4-fold between 2000-2014 and the current pipeline. The lack of progress in developing an effective disease modifying agent have been attributed to late intervention in advanced neuropathy and inadequate trial duration as well as the complex pathogenic mechanisms associated with DPN (Malik, 2016).

Figure 1.2. The proportion of disease modifying agents, symptom-reducing agents, diabetic foot agents and alternative interventions for DPN in 423 trials from February 1998 to June 2020 as shown on clinicaltrials.gov.



1.2.1 The role of biomarkers in drug development

Biomarkers provide direct or indirect evidence of the underlying pathology of the disease, reflect disease progression and identify the benefit of therapeutic intervention. Biomarkers have two significant roles in the process of drug development: 1. Identify disease for recruitment selection and 2. Determine drug efficacy as primary and secondary outcome measures.

Disease-modifying treatments are likely to respond better in early or mild neuropathy by intervening and halting pathological progression. Trials of patients with established DPN are destined to fail. Early DPN involves predominantly small fiber dysfunction/damage (Azmi et al., 2015, Divisova et al., 2012) and as neuropathy progresses large fiber neuropathy develops. Hence, recruiting patients with small fiber or asymptomatic neuropathy may allow evaluation of a drug in preventing DPN or repairing small nerve fiber damage. Composite scoring systems and assessments of large fiber neuropathy are useful to confirm the presence of mild DPN for recruitment and to assess the development of symptoms or disease progression as outcome measures. A review of disease modifying agents (n=42) in phase II and III trials revealed that

most of the trials were assessing large fiber neuropathy. Whilst 74% of trials undertook large fiber assessment using NCS, only 31% of trials assessed small fiber neuropathy using either IENFD or CCM.

Corneal confocal microscopy (CCM) is an ophthalmic imaging technique to quantify small fiber neuropathy (Petropoulos et al., 2013c, Petropoulos et al., 2014, Petropoulos et al., 2013a, Malik et al., 2003, Ahmed et al., 2012). It has been used as an outcome measure for DPN and other peripheral neuropathies in 22 trials from January 2018 to June 2020 as shown in Clinicaltrial.gov. Damage to corneal nerve fibers is associated with the severity of neuropathic symptoms (Kalteniece et al., 2020). CCM also has the ability to detect nerve repair at 6 months, whilst NCS and neuropathic symptoms improved after 24 months (Tavakoli et al., 2013, Azmi et al., 2019b). CCM can also predict DPN development (Pritchard et al., 2015, Lovblom et al., 2015, Edwards et al., 2017). Therefore, CCM has considerable merit to enrich trial cohorts, monitor neurodegeneration over time and reduce the length of trials that assess the effectiveness of disease modifying therapies.

IENFD was the gold standard as an objective measure for small fiber neuropathy (Lauria et al., 2010a, Lauria et al., 2010b). However, it's utility in clinical trials is limited due to it being an invasive and costly procedure, which does not allow repeated assessment of the same nerves. Whilst there is a correlation between IENFD and CCM measures, nerve fiber loss detected from different patients (Ziegler et al., 2014). The diagnostic accuracy expressed as the area under the receiver operating characteristic (ROC) curve for distinguishing small fiber neuropathy in patients with T2D from control subjects is 82% with corneal nerve fiber density (CNFD) and 66% with IENFD (Chen et al., 2015).

Nerve conduction studies (NCS) remain an essential technique due to widespread access and its objectivity and reproducibility for quantifying large fiber neuropathy (Bril et al., 1998). Decreases in sensory nerve action potential amplitude, sensory and motor nerve conduction velocity are associated with severity of neuropathic deficits (Dyck and O'Brien, 1989). Motor nerve conduction velocity (MNCV) can predict the development of diabetic foot ulceration (Carrington et al., 2002). However, the repeatability of NCS in trials varies considerably and may be responsible for the inability to identify benefits (Olney, 1998). NCS can vary

considerably if limb temperature is not maintained within a specified range throughout the test.

Quantitative sensory testing (QST) of warm and cold perception threshold have been shown to be reliable measures for small fiber neuropathy and vibration perception threshold for large fiber neuropathy (Azmi et al., 2019d, Dyck, 2014). However, QST is a subjective psychophysical test, dependent on patient motivation, alertness, and concentration and may not be sufficiently sensitive to detect a change in DPN (Malik, 2016).

Diabetic autonomic neuropathy (DAN) assessment is non-invasive and reproducible (Vinik et al., 2003). CAN assessment, which includes a battery of tests including DB-HRV, Valsalva maneuver and postural blood pressure change have been most used widely (Olney, 1998).

Composite scoring systems, including NDS (Young et al., 1993), mTCNS (Bril and Perkins, 2002b), NIS-LL (Bril, 1999), TNS-C (Cornblath et al., 1999), MDNS coupled with the MNSI (Feldman et al., 1994) and UENS (Singleton et al., 2008) are weighted predominantly to the assessment of large fiber neuropathy (Zilliox et al., 2015), which clearly limits their utility to assess small fiber neuropathy. These composite scoring systems show association with large fiber assessments such as NCS and VPT but no association with IENFD at the distal leg or thigh or cold perception threshold (Zilliox et al., 2015).

1.2.2 The contribution of this PhD thesis to assess the effect of glucose lowering therapies on neuropathy using CCM as a primary outcome measure

Both glucagon-like peptide 1 (GLP-1) receptor agonists (Kan et al., 2012, Himeno et al., 2011) and thiazolidinediones (TZDs) (Qiang et al., 1998, Pop-Busui et al., 2013, Yamagishi et al., 2008, Wiggin et al., 2008) produce a durable reduction in HbA1c (Abdul-Ghani et al., 2017). GLP-1 receptor agonists stimulate insulin secretion in response to hyperglycemia, delay gastric emptying leading to weight loss and inhibit hepatic glucose production. TZDs are potent insulin sensitizers and improve β-cell function.

There are conflicting data regarding the beneficial effect of GLP-1 receptor agonists on DPN. Preclinical studies showed that exendin-4, a GLP-1 receptor agonist prevents sensory (Kan et al., 2012) and motor nerve conduction slowing (Himeno et al., 2011) and a reduction in IENFD in T1D mice. However, exenatide showed no effect on the incidence of DPN, cardiovascular

autonomic neuropathy (CAN) or IENFD in patients with T2D over 18 months (Jaiswal et al., 2015). Liraglutide failed to show a benefit on DAN or sensory and motor nerve conduction in 39 patients with T1D and established DPN (Brock et al., 2019).

There is evidence showing that TZDs might have a neuroprotective effect. In preclinical studies, troglitazone prevented nerve conduction slowing and maintained normal myelinated fiber architecture and density in T1D rats (Qiang et al., 1998). Pioglitazone prevented nerve conduction slowing and reduced macrophage infiltration in the sciatic nerve in T1D rats (Yamagishi et al., 2008). Rosiglitazone prevented thermal hypoalgesia and reduced oxidative stress in the sciatic nerve of T1D mice (Wiggin et al., 2008). In the BARI 2D trial (Pop-Busui et al., 2013), rosiglitazone significantly reduced the 4-year cumulative incidence of DPN compared to insulin treatment in patients with T2D. The neuroprotective effect of TZDs may be attributed to a reduction in oxidative stress and advanced glycated end products.

Chapter 8 has assessed the effect of combination treatment of exenatide and pioglitazone or basal-bolus insulin on DPN measures in patients with poorly controlled T2D over a 1-year period. DPN was measured using CCM as a primary outcome measure and DN4 questionnaire, vibration perception threshold (VPT) and sudomotor function as secondary outcome measures. This study also evaluated the effect of the treatments on diabetic retinopathy. This is a sub-study of the Qatar study (Abdul-Ghani et al., 2017), an open-label, randomized controlled trial, which showed a rapid and effective reduction in HbA1c after treatment with the combination treatment or basal-bolus insulin in patients with poorly controlled T2D.

1.3 Application of CCM as a biomarker of neurodegeneration in dementia

CCM was originally pioneered to identify neurodegeneration in DPN (Petropoulos et al., 2013c, Petropoulos et al., 2014, Petropoulos et al., 2013a, Malik et al., 2003, Ahmed et al., 2012) and subsequently in a range of other peripheral neuropathies (Petropoulos et al., 2019) and in a large group of healthy people (Tavakoli et al., 2010). It generates *in vivo* images of the sub-basal nerve plexus from which corneal nerve morphology is analysed using validated image analysis software (Dabbah et al., 2011) which reduces inter- and intra-rater variability and enables objective quantification of the corneal nerve morphology (Vagenas et al., 2012, Petropoulos et al., 2013c, Kalteniece et al., 2017). CCM has also been used to identify corneal

nerve degeneration in a number of central neurodegenerative diseases, including Parkinson's disease (Kass-Iliyya et al., 2015, Podgorny et al., 2016), amyotrophic lateral sclerosis (Ferrari et al., 2014) and multiple sclerosis (Petropoulos et al., 2017, Bitirgen et al., 2017b, Mikolajczak et al., 2016). However, the association between corneal nerve fiber pathology and neurodegeneration in dementia has not been studied.

1.3.1 Biomarkers for Alzheimer's disease

Dementia is a progressive neurodegenerative disorder currently affecting 40-50 million people worldwide (Wu et al., 2017, Prince et al., 2013). Therapeutic and psychological interventions for people with early stage dementia can improve cognition, independence, and quality of life (Prince et al., 2011). However, the diagnosis of mild cognitive impairment (MCI) or early dementia can be challenging due to the insidious onset of disease and gradual cognitive decline. A diagnosis of MCI requires a change in cognition, evidence of impairment in at least one cognitive domain but with preserved ability to function independently in daily life (McKhann et al., 2011). However, cognitive assessment tests are influenced by age, educational and cultural background (Albert et al., 2011). Biomarkers that allow for greater diagnostic certainty to distinguish normal cognition due to aging from MCI and dementia are required.

The 2011 National Institute on Aging and the Alzheimer's Association (NIA-AA) diagnostic criteria for Alzheimer's disease (AD) (Albert et al., 2011) included imaging and cerebrospinal fluid (CSF) biomarkers to confirm the diagnosis of AD, but not to predict the development of AD in patients with MCI or early dementia (McKhann et al., 2011). The 2018 revision of the NIA-AA diagnostic criteria has changed the role of biomarkers from confirming the presence of AD to identifying the disease in its asymptomatic stages (Jack et al., 2018). Based on longitudinal studies, the NIA-AA proposed the A/T/N classification system for AD biomarkers, in which A is for amyloid beta (Aß) biomarkers, T for tau biomarkers and N for neurodegeneration biomarkers. Biomarkers for Aß and tau include positive Aß deposition in the brain utilizing amyloid positron emission tomography (PET) imaging (Forsberg et al., 2008, Grimmer et al., 2013), reduced CSF Aß42 or Aß42/Aß40 ratio and increased CSF tau protein concentrations (Mattsson et al., 2009). Biomarkers for neurodegeneration include reduced [^{18}F] fluorodeoxy-glucose (FDG) uptake on PET in the temporoparietal lobes reflecting

reduced brain activity (Landau et al., 2010) and hippocampal or medial temporal lobe atrophy (MTA) on MR imaging (Jack et al., 1999, Bouwman et al., 2007).

1.3.2 Limitations of current biomarkers for Alzheimer's disease

NIA-AA proposed biomarkers are relatively accurate, objective and reliable but have limitations. Amyloid-PET reflects aggregated Aß within the brain, a neuropathological hallmark of AD. However, amyloid-PET is costly and has health hazards because of exposure to radioactivity (Clark et al., 2011). Furthermore, the prevalence of amyloid PET positivity among elderly subjects without cognitive impairment is also high and is a major limitation that reduces diagnostic utility (Jansen et al., 2015). Amyloid deposition can occur decades before the manifestation of clinical symptoms (Villemagne et al., 2013) and the degree of amyloid deposition does not correlate with cognitive decline in AD (Khosravi et al., 2019). There are also practical concerns for amyloid-PET analysis due to variations in protocols and a lack of standardized cut-off values for interpretation (Suppiah et al., 2019). FDG-PET is a widely available radiotracer with established cutoff values expressed as standardized uptake values (SUVs) for disease process quantification (Suppiah et al., 2019). However, FDG PET has low accuracy in late-onset AD (≥74 years of age) (Ng et al., 2007) and has no significant association with regional Aß deposition in the brain (Altmann et al., 2015).

CSF biomarkers can identify AD in its asymptomatic stages as metabolic and other pathological alterations occurring in the brain can be detected in the CSF. However, there are conflicting reports regarding the concentration of these proteins needed to accurately predict the development of AD in patients with MCI (Lee et al., 2019). Accessing CSF requires a lumbar puncture which is invasive, can be painful and is not routinely undertaken.

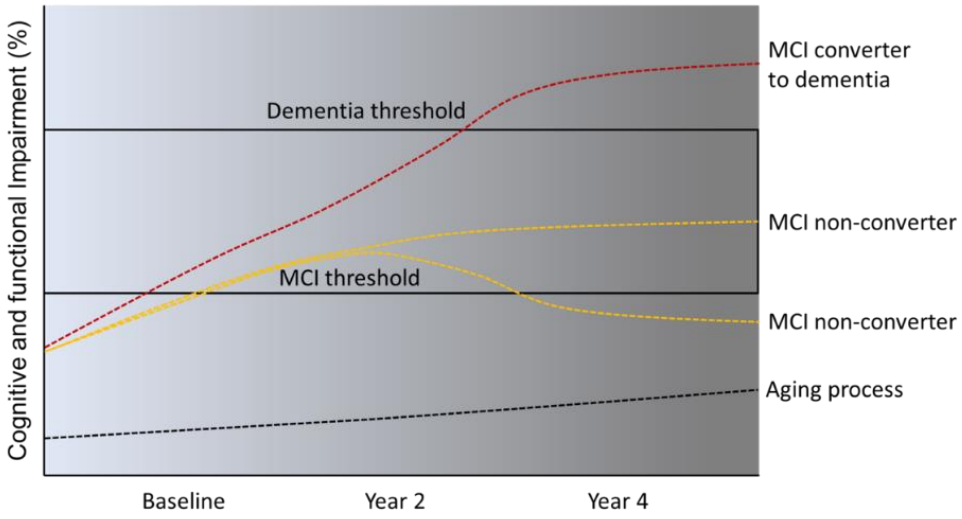
Structural MR imaging for brain atrophy has high diagnostic accuracy for patients with late-onset AD (Duara et al., 2008, Heo et al., 2013, Cavedo et al., 2014). However, it has poor accuracy for distinguishing patients with MCI or early-onset AD from subjects without cognitive impairment (Falgas et al., 2019).

1.3.3 Areas for improvement in biomarkers for Alzheimer's disease

As evidenced by the recent diagnostic accelerator program call from the Alzheimer's Drug Discovery Foundation (ADDF), there is a need for accurate and reliable biomarkers that are accurate, non-invasive, simple to perform and inexpensive. Bill Gates and the ADDF have committed $30 million to the diagnostic accelerator program to develop biomarkers to better understand how the disease progresses, more easily identify people for clinical trials, and more accurately monitor their response to treatments.

The efficiency of a biomarker for AD is evaluated based on its ability to: 1) provide direct or indirect evidence of the underlying pathology of the disease; 2) identify subtypes of MCI which do or do not progress to dementia (Figure 1.3), and 3) reflect disease progression and/or identify the benefit of therapeutic intervention. For a biomarker to be accepted as a pathologic, prognostic and/or monitoring marker it must be accurate with sensitivity and specificity equal to or higher than 80% (Thies et al., 1999). Biomarkers that have plateaued to maximal impairment or have not shown significant changes (ceiling and floor effects, respectively) are poor markers.

Figure 1.3. A theoretical model of progressive cognitive and functional impairment in a patient with MCI that may reverse back to normal cognitive function, remain with MCI or progress to dementia.



1.3.4 The contribution of this PhD thesis in assessing the association of corneal nerve morphology with central neurodegeneration in MCI and dementia

Chapter 9 has determined whether there is significant corneal nerve fiber loss in patients with MCI and dementia compared to age-matched controls and the association between corneal nerve fiber measures with cognitive function and functional independence.

1.3.5 The impact of diabetes in dementia

A large body of data shows that diabetes has a major influence on corneal nerve pathology (Petropoulos et al., 2013c, Petropoulos et al., 2014, Petropoulos et al., 2013a, Malik et al., 2003, Ahmed et al., 2012). Epidemiological studies also show that individuals with T2D have an increased risk of dementia (Zhang et al., 2017a, Gudala et al., 2013); the relative risk for AD and vascular dementia (VaD) for people with diabetes compared to people without diabetes is 1.53 (95% CI 1.42-1.63) (Zhang et al., 2017a) and 2.27 (95% CI 1.94-2.66) (Gudala et al., 2013), respectively. This increased risk of dementia in patients with T2D is attributed to non-AD mechanisms of neurodegeneration as T2D is not associated with excess Aß plaques and neurofibrillary tangles of hyperphosphorylated tau protein in the brain (Abner et al., 2016, Dos Santos Matioli et al., 2017). However, patients with T2D have a 1.57-times increased odds of an infarct, and 1.71-times increased odds of lacunes, small subcortical infarcts in the brain (Abner et al., 2016). Infarcts and lacunes double the risk of dementia occurring within 5 years (Vermeer et al., 2003) and could decrease cognitive reserve in patients who have accumulating plaques and tangles (Snowdon et al., 1997).

1.3.6 Brain atrophy measurement on MRI: an established biomarker for neurodegeneration in Alzheimer's disease

Brain atrophy measurement on MRI is an established biomarker for neurodegeneration in Alzheimer's disease (AD) but not for MCI or dementia (Albert et al., 2011, McKhann et al., 2011). Brain atrophy occurs as the result of dendritic, myelin and axonal loss (Frisoni et al., 2010). There is progressive medial temporal lobe atrophy (MTA) in subjects with MCI and dementia compared to those with no cognitive impairment (NCI) (Du et al., 2001, Urs et al., 2009). MTA rating has been shown to have high diagnostic accuracy for probable (Thies et al.,

1999) and established AD (Heo et al., 2013, Cavedo et al., 2014) but cannot distinguish patients with MCI or early-onset AD from subjects with NCI (Falgas et al., 2019). MTA has been reported in patients with VaD (Barber et al., 2000, Cho et al., 2009).

1.3.7 The contribution of this PhD thesis in assessing the diagnostic ability of CCM for MCI and dementia compared with MRI

Chapter 10 has compared the diagnostic accuracy of CCM with MTA rating for MCI and dementia, including AD, VaD and mixed AD. MTA was quantified in T1-weighted 3D MPRAGE MRI using the Duara visual rating (Duara et al., 2008). Given that diabetes is a confounding factor, the effect of diabetes on CCM measures in MCI and dementia was also assessed.

It is important to assess the diagnostic ability of CCM not only for pure AD but also VaD and combined AD with vascular lesions. This is because autopsy studies have reported that amyloid deposition and vascular lesions in the brain are the most frequent pathologies present concurrently in patients with MCI and dementia (Jellinger and Attems, 2007, Schneider et al., 2007, Schneider et al., 2009). Vascular lesions are present in approximately 50% of patients diagnosed with AD, even in clinical trials of subjects who have been extensively screened for pure AD (Wang et al., 2012). Whilst AD is considered to be the most common type of dementia accounting for 60-80% of cases (Hebert et al., 2013) followed by VaD in approximately, 10% of cases (Fernando et al., 2004), in Qatar the prevalence of VaD was 36% (Anoop Sankaranarayanan, 2016). CCM has shown corneal nerve loss in patients with TIA and minor (Gad et al., 2019) as well as major ischemic stroke (Khan et al., 2018) and corneal nerve loss has been associated with the presence of white matter hyperintensities, independent of the presence of diabetes (Kamran et al., 2020).

1.4 PhD thesis submission in publication format

The author has been granted permission to submit this PhD thesis in a publication format by his Director of Studies Professor Mark Slevin, First Supervisor Dr. Christopher Murgatroyd, Mentor Professor Rayaz A. Malik and approved under the Manchester Metropolitan University, Faculty of Science and Engineering and Faculty Research Degrees regulations. All chapters have been submitted for publication apart from chapters 1, 2 and 11, which are the introduction, methods and conclusion, respectively. Chapter 3 was published in

Diabetes/Metabolism Research and Reviews on 30/12/19. Chapter 4 was published in the Journal of Diabetes Investigation on 3/3/19. Chapter 5 was accepted for publication in the Journal of Diabetes Investigation on 16/7/20. Chapter 6 was published in the American Journal of Hypertension on 23/04/2019. Chapter 7 was published in Frontiers of Endocrinology on 25/05/2019. Chapter 8 was published in BMJ Open Diabetes Research Care on 13/5/20. Chapter 9 was published in Annals of Clinical and Translational Neurology on 6/2/19. Chapter 10 was accepted for publication in Journal of Alzheimer's Disease on 20/7/20.

Chapter 2: Experimental Design and Methods

2.1 Project plan and ethical approvals

This PhD started on September 27th, 2017 and the submission deadline of the PhD thesis was on September 25th, 2020 (Figure 2.1). All clinical research activities done in Doha, Qatar were approved by the Weill Cornell Medicine in Qatar (WCM-Q) IRB (Ref. # 15-00078, 13-0076, 15-00019), Hamad Medical Corporation (HMC) IRB (Ref. # 16324/16, IRB#: 13-0076, RP14494/14), Manchester Metropolitan University (MMU) ethics committee (EthOS ref. # 0565). The research work for Chapter 6 done at the NIHR Wellcome Trust Clinical Research Facility in Manchester, UK was approved by the NRES Committee North West - Greater Manchester West (REC Ref. # 09/H1006/38). All subjects gave informed consent to take part in the study. The research adhered to the tenets of the declaration of Helsinki.

Subject recruitment started before the starting date of the PhD study and completed in September 2017 for Chapter 6 and 7, November 2018 for Chapter 8, February 2019 for Chapter 3, 4 and 5, and July 2019 for Chapter 9 and 10. Data collection and analysis were conducted throughout the study.

2.2 Contribution and management of this PhD project

Figure 2.2 outlines the five components used to manage this PhD: 1. Management of the studies throughout the course of my PhD, 2. Screening and recruiting subjects, 3. Performing study procedures including assessments, data collection and analysis, 4. Dissemination of results through presentation and publication and 5. Communication and coordination to ensure completion of all tasks. Table 2.1 shows the contribution and management of this PhD. The contribution of the PhD. student described in detail in Table 2.1 was the same in all chapters.

Figure 2.1. PhD project timeframe.

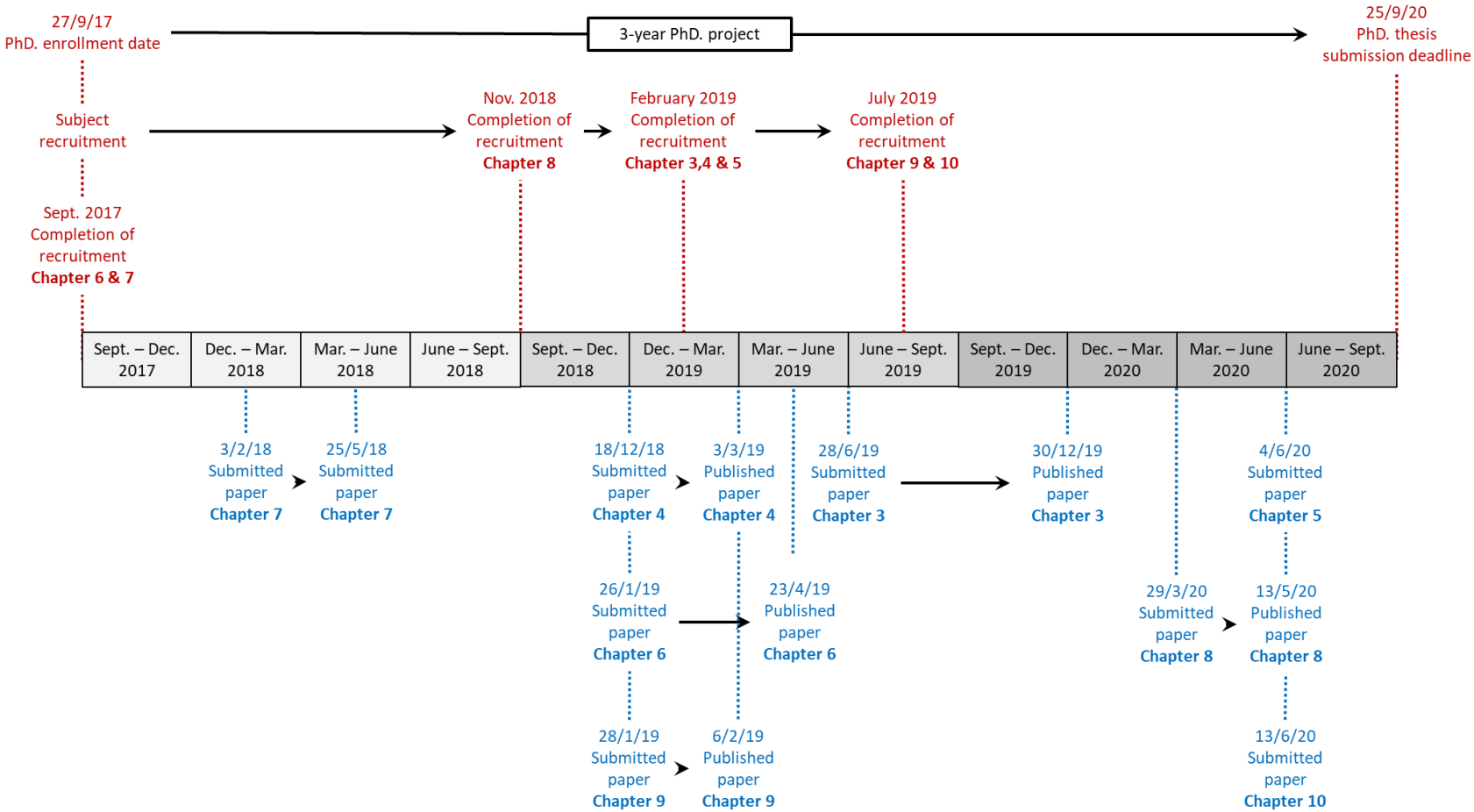


Figure 2.2. Flow chart showing the management of this PhD.

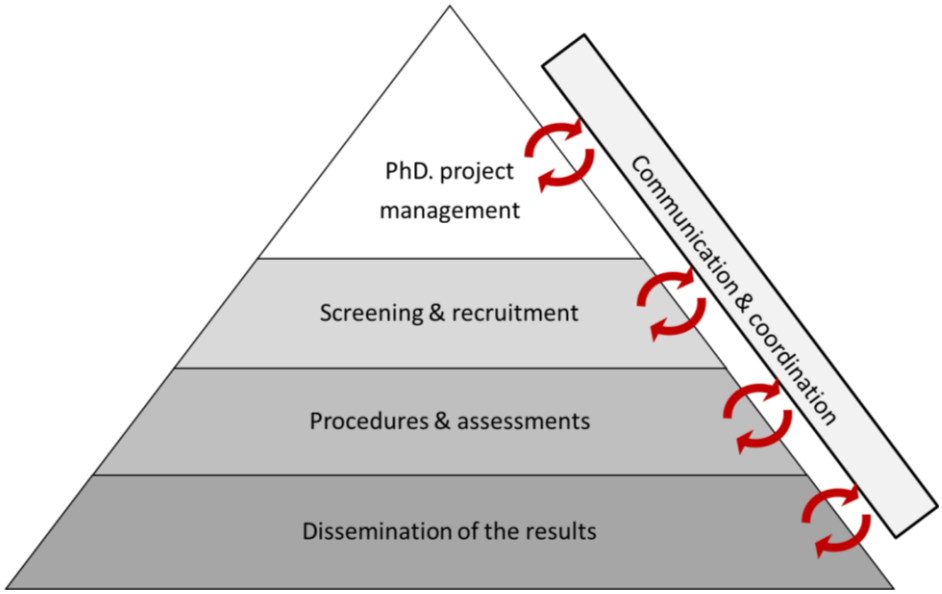


Table 2.1. Contribution and management of this PhD.

PhD project management		
Investigators	**Role**	**Tasks**
Prof. Rayaz Malik	PhD. mentor	Offered PhD, secured funding for the PhD programme, provided resources and personnel for the clinical work of this PhD, oversaw the PhD project and provided guidance.
Prof. Mark Slevin	Director of PhD study	Oversaw the PhD project and provided guidance.
Dr. Chris Murgatroyd	PhD supervisor	Oversaw the PhD project and provided guidance.
Georgios Ponirakis	PhD student	Generated, collated and analysed data and wrote up for publication.
Dr. Hanadi Al Hamad	Co-investigator	Oversaw all the research activities in Rumailah Hospital.
Dr. Ziyad Mahfoud	Statistician	Oversaw all statistical analyses.
Screening and recruitment		
Investigators	**Role**	**Tasks**
Physicians	Co-investigators	Screened and recruited
Communication & coordination		
Investigator	**Role**	**Tasks**
Georgios Ponirakis	PhD student	Ensured everyone involved in the studies understood the objectives and were aware of their responsibilities, regularly updated my mentor, PhD Director and supervisor on study progress, recruitment, any issues and study results.
Project procedures		
Investigators	**Role**	**Tasks**
Georgios Ponirakis	PhD student	Consented subjects, performed cognitive function assessments for Chapter 9 & 10, CCM for Chapter 6, 8-10 and neuropathy assessments, data collection and statistical analysis.
Physicians & nurses	Co-investigators	Performed diagnosis (T2D, normal cognition, MCI, dementia), consented subjects, performed cognitive function assessment, MRI brain interpretation and atrophy analysis, and neuropathy assessments.
Dissemination of the results		
Investigators	**Role**	**Tasks**
Georgios Ponirakis	PhD student	Presented the work in national and international conferences and wrote up papers.
Prof. Rayaz Malik	Mentor	Reviewed and revised PhD thesis, papers, presentations.
Prof. Mark Slevin	Director of PhD. study	Reviewed and revised PhD thesis, papers, presentations.
Dr. Chris Murgatroyd	PhD. supervisor	Reviewed and revised PhD thesis, papers, presentations.

2.3 Study sites

All the research work for this PhD was conducted in Qatar, apart for Chapter 6, which was done in Manchester, UK.

In **Weill Cornell Medicine in Qatar (WMC-Q)**, I worked on the Institutional Review Board (IRB) applications and amendments, submitted conflict of interests, performed data quality and analysis, wrote papers and my PhD thesis.

In **primary health care (PHC) centres** of **Umm Ghuwailina, Al Khor, Al Daayen and Al Rayyan** and the National Diabetes centres in Qatar, **Hamad General Hospital** (**HGH**) and **Al-Wakra Hospital**, subjects with T2D were screened, recruited, consented and assessed.

In **Rumailah Hospital**, subjects with no cognitive impairment (NCI), mild cognitive impairment (MCI), dementia with and without T2D were screened, recruited, consented and assessed.

In the **Manchester Diabetes Centre, Manchester Royal Infirmary**, subjects with T1D and control subjects were screened and recruited, and in the **NIHR Wellcome Trust Clinical Research Facility** in Manchester, UK, subjects with T1D and control subjects were screened, recruited, consented and assessed.

2.4 Project population

The recruitment selection, inclusion and exclusion criteria for each study are described in detail in the methods section in each chapter. There were some common exclusion criteria in all chapters:

- Other causes of peripheral neuropathy than diabetes such as vitamin-B_{12}, folate deficiency, hypothyroidism and severe kidney impairment.
- Unable to undergo CCM assessment due to lack of cooperation.
- Unable to understand English or Arabic.
- Unable to write or sign the consent/assent form.
- Pregnant women and prisoners.

For studies that included CCM assessments:

- Ineligible for CCM assessments due to history of ocular trauma or previous ocular surgery in the preceding six months, corneal dystrophy, severe dry eyes or allergic reactions to local eye anesthetic.

2.5 Recruitment method

Subjects were screened for eligibility and invited for the study by clinicians involved in the study on the day they attend the clinic. Eligible subjects were required to give informed written consent to take part in the study.

2.6 Procedures and assessments

2.6.1 Demographic, clinical and metabolic characteristics and list of medications

Gender, ethnicity, age, duration of diabetes and body mass index (BMI) were recorded. The average systolic (SBP) and diastolic blood pressure (DBP) of two readings were obtained from the subject's left arm while seated with the arm at heart level, using a standard zero mercury sphygmomanometer after 10-15 minutes of rest. A non-fasting blood sample was collected through venepuncture from each subject into EDTA tubes and transported within 2 hours to a central certified laboratory at HGH. Glycated hemoglobin (HbA1c), total cholesterol and triglyceride, vitamin B_{12} and folate, thyroid stimulating hormone (TSH), free thyroxine (FT4) were recorded from Cerner, Hamad Medical Corporation electronic medical records. Poor glycemic control was defined as HbA1c ≥9%. Hypertension was defined as average SBP ≥140 mmHg and/or the use of anti-hypertensive medication, as per WHO/ISH Guidelines (Moser, 1999). Hyperlipidemia was defined as a total cholesterol level ≥6.2 mmol/L and/or triglyceride level of ≥2.3 mmol/L or if the patient was treated with a statin. Obesity was classified according to WHO criteria with a BMI ≥30 kg/m^2 (Report of a WHO consultation, 2000). Current cigarette smoking was defined as having smoked at least one cigarette every day for ≥1 year preceding the study visit. Physical activity was defined as doing some physical activity including walking for ≥30 minutes/day, at least 3 times a week over the last year.

2.6.2 Assessment of diabetic neuropathy and painful neuropathy (Chapter 3-7)

The diagnosis of DPN for the studies done in Qatar was based on the presence of one or more neuropathic symptoms such as burning pain, painful cold, electric shocks, tingling, pins and needles and numbness, and impaired vibration perception threshold (VPT) in the feet. VPT was measured by a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK) (Figure 2.3) on the pulp of the large toe on both feet and the average value of three measurements was recorded in Volts (V) ranging from 0 - 50V. A VPT ≥15V was defined as impaired vibration perception consistent with the presence of DPN (Wiles et al., 1991) and a VPT ≥25V as high risk for diabetic foot ulceration (DFU) (Young et al., 1994).

The diagnosis of DPN in Chapter 6 was according to the criteria established by the Toronto Diabetic Neuropathy Expert Group (Tesfaye et al., 2010). These criteria include neuropathy symptoms or neuropathy signs and an abnormality of NCS or a validated measure of small fiber neuropathy (corneal nerve fiber length) (Petropoulos et al., 2013a, Chen et al., 2015). Neuropathic symptoms were assessed using the DNS score (Meijer et al., 2002), a four-item validated symptom score for symptoms of unsteadiness in walking, neuropathic pain, paraesthesia, and numbness, giving a maximum score of 4 points, with a score of ≥ 1 defining the presence of neuropathic symptoms. Neuropathy signs were defined using the NDS (Young et al., 1993) that includes examination of vibration perception using a 128-Hz tuning fork, pin-prick on the tip of the large toe, temperature perceptions in the dorsum of the feet, and the presence or absence of ankle reflexes. Subjects scoring > 2/10 were considered to have signs of neuropathy. The techniques to assess for symptoms and signs of DPN in chapter 6 were different to Chapter 3-5 because it was part of the LANDMark study that preceded my PhD. VPT assessment used for Chapter 3-5 is a subjective psychophysical test, dependent on patient motivation, alertness, and concentration (Malik, 2016), whereas CCM (Petropoulos et al., 2013c, Petropoulos et al., 2014, Petropoulos et al., 2013a, Malik et al., 2003, Ahmed et al., 2012) and NCS (Bril et al., 1998) used for Chapter 6 are objective measures of DPN and have high reproducibility for quantifying small and large fiber neuropathy, respectively.

For the studies done in Qatar, pDPN was assessed using the Douleur Neuropathique en 4 (DN4) questionnaire in Arabic and English as previously described (Azmi et al., 2019c). Previously diagnosed DPN and pDPN were self-reported. For Chapter 6, pDPN was defined by

a combination of deficits with an NDS score >2 and the presence of painful symptoms using the McGill Pain Questionnaire (Melzack, 1975).

Figure 2.3. Neurothesiometer devices used to assess for impaired vibration perception threshold. Georgios Ponirakis (PhD student) in the photo.

2.6.3 Corneal confocal microscopy (CCM) image acquisition (Chapter 6, 8-10)

CCM analysis was performed with the Heidelberg Retinal Tomograph III Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany) (Figure 2.4). This device uses a 670 nm red wavelength diode laser, which is a class I laser and therefore does not pose any ocular safety hazard. A 63x objective lens with a numerical aperture of 0.9 and a working distance, relative to the applanating cap (TomoCap, Heidelberg Engineering GmbH, Heidelberg, Germany) of 0.0 to 3.0 mm was used. The images produced using this lens are 384 µm × 384 µm with a 15° × 15° field of view and 10 µm/pixel transverse optical resolution.

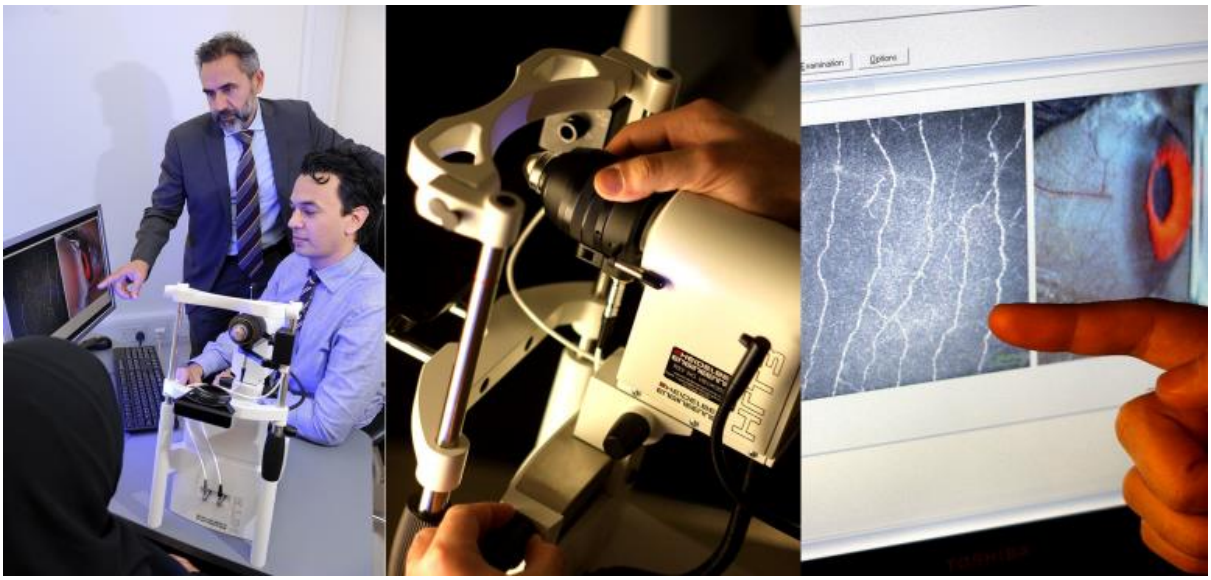
The cornea was locally anesthetized by instilling 1 drop of 0.4% benoxinate hydrochloride (Chauvin Pharmaceuticals, Chefaro, UK) and Viscotears (Carbomer 980, 0.2%, Novartis, UK) was used as the coupling agent between the cornea and the TomoCap as well as between the TomoCap and the objective lens. Subjects were asked to place their chin on the chin rest and press their forehead against the forehead support. They were asked to fixate with the eye not being examined on an outer fixation light to enable examination of the central cornea. Images

of the sub-basal nerve plexus were captured using the “section” mode. Multiple images were taken from the sub-basal nerve plexus.

2.6.4 CCM image extraction and analysis

CCM image extraction was performed at a separate time by the PhD student, Georgios Ponirakis who was blinded to patient diagnosis. Three to five representative sharp images of the sub-basal nerve plexus were selected per eye by filtering out blurred images or pressure lines caused by the pressure applied between the TomoCap and cornea or out of focus images. Manual CCM image analysis was performed using CCMetrics, a validated image analysis software (Dabbah et al., 2011). Corneal nerve morphology was quantified as corneal nerve fiber density (CNFD, fibers/mm^2), branch density (CNBD, branches/mm^2) and fiber length (CNFL, mm/mm^2).

Figure 2.4. The Heidelberg Retinal Tomograph III device coupled with the Rostock Cornea Module used for corneal confocal microscopy (CCM). Prof. Rayaz Malik (PhD mentor) on the far right and Georgios Ponirakis (PhD student) performing CCM analysis in the photo.



2.6.5 Intraepidermal nerve fiber density (Chapter 6)

A 3 mm punch skin biopsy was taken from the dorsum of the left foot under 1% lidocaine local anaesthesia. Skin samples were immediately fixed in 4% (wt/vol.) paraformaldehyde for 24 hours and then cryoprotected in sucrose, frozen and cut into 50 μm sections. Immunohistochemistry was performed as previously described (Azmi et al., 2015). A Zeiss AxioImager M2 microscope (Carl Zeiss, Jena, Germany) was used to quantify intra epidermal

nerve fiber density (IENFD), which is the total number of nerve fibers per millimetre length of epidermis (no./mm), in accordance with established criteria (Lauria et al., 2010b).

2.6.6 Autonomic neuropathy (Chapter 6)

Cardiovascular autonomic neuropathy (CAN) was evaluated using the ANX 3.0 autonomic nervous system monitoring device (ANSAR Medical Technologies Inc. Philadelphia, US) (Orlov et al., 2012). Deep Breathing-Heart Rate Variability (DB-HRV) was assessed by R-R interval variation via surface electrodes over 1 minute at a frequency of 6 breaths/minutes.

Sudomotor dysfunction was assessed using the Neuropad plaster (Miro Verbandstoffe, Wiehl-Drabenderhöhe, Germany) applied to the plantar aspect of the 1st metatarsal head for 10 minutes, followed by quantification of the percentage colour change of the Neuropad (Ponirakis et al., 2014).

2.6.7 Quantitative sensory testing for warm and cold perception (Chapter 6)

Quantitative sensory testing (QST) included measurement of warm and cold perception thresholds (WPT & CPT) on the dorsum of the left foot using the method of limits with the MEDOC *TSA II* (Medoc Ltd. Ramat Yishai 30095, Israel).

2.6.8 Nerve conduction studies (NCS) (Chapter 6)

NCS were undertaken using a Dantec "Keypoint" system (Dantec Dynamics Ltd. Bristol, UK) equipped with a DISA temperature regulator to keep lower limb temperature constantly between 32 and 35^{o}C. Sural nerve action potential (SNAP), sural nerve conduction velocity (SNCV), tibial compound motor action potential (TCMAP), tibial motor nerve conduction velocity (TMNCV), peroneal compound motor action potential (PCMAP) and peroneal motor nerve conduction velocity (PMNCV) were assessed in the right lower limb by a consultant neurophysiologist. Sural sensory responses were measured using a bipolar bar electrode (inter-electrode distance 3 cm) attached over the sural nerve at the lateral malleolus. Stimulation was performed 140 mm proximal to the active recording electrode in the calf. Abnormal nerve conduction was defined based on two abnormal nerve conduction velocities

of either SNCV, TMNCV or PMNCV. The cut-off values of the nerve conduction velocities were defined on the – 2 SD from the mean based on our control population.

2.6.9 Diagnosis of normal cognition, MCI and dementia (Chapter 9 & 10)

The diagnosis of MCI and dementia was based on the ICD-10 criteria (International Advisory Group for the Revision of and Behavioural, 2011). A joint consultative model in the Department of Geriatric Medicine run by geriatricians and geriatric psychiatrists with advice and consultation from the neurologists was applied to ensure the correct diagnosis, especially to exclude reversible, complex and young-onset dementia. The diagnosis of MCI and dementia was based on a patient history and examination, which includes (1) presenting complaint and history of illness; (2) comprehensive history of each of the cognitive domains; (3) psychiatric history for ruling out depression, mood disorders, and psychosis; (4) medical history including episodes of delirium and other medical comorbidities; (5) medication history; (6) functional history of basic daily living activities; (7) components of comprehensive geriatric assessment; (8) detailed psychiatric mental status examination and cognitive screening using MoCA. A comprehensive organic work-up including blood investigations and brain imaging was undertaken to exclude other potentially reversible causes of cognitive decline such as tumors, subdural hematoma or normal pressure hydrocephalus. The final diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI) and dementia was made according to consensus decision by geriatricians, geriatric psychiatrists and neurologists. The diagnosis of AD was based on radiological evidence, including volume loss of hippocampi, entorhinal cortex, and amygdala on MRI as described by Dubois et al. (Dubois et al., 2009). Diagnosis of probable or possible vascular dementia (VaD) was based on the NINDS-AIREN criteria (Roman et al., 1993), which specifies evidence of cerebrovascular disease by brain MRI, including multiple large vessel infarcts, a single strategically placed infarct (angular gyrus, thalamus, basal forebrain, posterior [PCA] or anterior cerebral artery [ACA] territories), multiple basal ganglia white matter lacunes, extensive periventricular white matter lesions, or combinations thereof. Mixed dementia was based on AD combined with evidence of vascular lesions in the brain.

2.6.10 Cognitive screening (Chapter 9 & 10)

Cognitive function was assessed by the occupational therapist using the Montreal cognitive assessment (MoCA) Arabic and English version. The MoCA assesses seven cognitive domains including visuospatial/executive, naming, memory, attention, language, abstraction and delayed recall giving a total score of 30. A score of $\leq$ 26 indicates cognitive impairment (Nasreddine et al., 2012). Educational level was recorded. A point was added for individuals with formal education below 6th grade. Cognitive symptom duration was estimated from the clinical history obtained from the participant and family members.

2.6.11 Functional independence screening (Chapter 9 & 10)

The Functional Independence Measure (FIM) was administered by the occupational therapist. FIM is an 18-point screening test of which 13 are for motor and 5 for cognitive function. Each point was scored from 1 to 7. The total FIM score ranges from 18 to 126. There is no cut-off point for FIM, but a high score indicates greater independence (Talmelli et al., 2013).

2.6.12 Brain MRI acquisition (Chapter 10)

MRI was performed on a superconductive magnet operated at 3T (Skyra, Siemens) at the MRI unit in Rumailah Hospital (Figure 2.5). The subject's head was immobilized with a head holder to minimize motion artifacts. A T1-weighted 3D magnetisation prepared rapid acquisition gradient echo sequence (MPRAGE) was obtained in the sagittal plane with a 1 mm slice thickness, repetition time of 1900 ms, echo time of 2.67 ms and 2.46 ms, inversion time of 1100 ms and 900 ms, flip angle of 9 degree and 15 degree, and FOV= 240 x 100. Coronal and axial reformatted MPRAGE images were reconstructed from the sagittal 3D sequence.

2.6.13 Medial temporal lobe atrophy visual rating (Chapter 10)

T1-coronal images at the level of the midbrain were used to score for right and left medial temporal lobe atrophy (MTA). The right and left hippocampi, entorhinal cortices, perirhinal cortices were separately rated by a certified neuroradiologist according to the five-point scale developed and validated by Duara et al, and a combined visual MTA score for each hemisphere was calculated averaging the three measurements (Duara et al., 2008). The coronal reformatted MRI slice at the level of the mammillary bodies seen in the sagittal plane was used to define the outline of the medial temporal lobe. The outline of the entorhinal

cortex in this slice was defined by the anterior parahippocampal gyrus and adjacent white matter (seen medial to the collateral sulcus and inferior to the hippocampus). The outline of the perirhinal cortex was defined by the fusiform gyrus and adjacent white matter (seen lateral to the collateral sulcus and medial to the occipitotemporal sulcus).

Figure 2.5. MRI brain performed on a superconductive magnet operated at 3T (Skyra, Siemens) at the MRI unit in Rumailah Hospital.

2.7 Data management

All data were stored in a spreadsheet in password protected and encrypted computers in Rumailah Hospital and WCM-Q. Data are to be retained for 3 years after project completion as it is an IRB requirement.

2.8 Statistical analysis

Statistical analysis applied to the specific study are described in detail in the methods section in each chapter.

Patients' demographic and clinical characteristics were summarized using means and standard deviations for numeric variables and frequency distribution for categorical variables. Continuous parametric variables were compared using unpaired t-test or non-parametric variables when the distribution had skewness of < -1 or > 1 with Mann-Whitney test.

Categorical variables were compared using Chi-squared test or Fisher's exact test when expected cell counts fell below 5. Changes between baseline and 1-year follow-up were compared using a paired t-test.

Multiple linear regression analysis was performed for continuous dependent variables and included all variables with P≤0.05 at the bivariate level. Residual plots were used to determine for linearity, normality, constant variance, and independence. The regression coefficient (beta) and the corresponding 95% confidence intervals (95% CI) are presented.

Multiple logistic regression analysis was performed for categorical dependent variables and included all variables with p-value of 0.10 or less at the bivariate level. Adjusted odds ratios and their corresponding 95% confidence intervals are presented.

Receiver operating characteristic (ROC) curve analysis was used to determine the ability of CNFD, CNBD and CNFL to distinguish patients with MCI and dementia from healthy controls. The area under curve (AUC), and two cut-off point with the maximal sum of sensitivity and specificity was calculated.

A two-tailed P value of ≤0.05 was considered significant.

2.9 Risk assessment and mitigation

The risk of recruitment delays was low since an established system of recruitment was in place before the start of the PhD project.

For CCM assessment, drops used to numb the eyes may cause some mild discomfort, which should subside within ~5-10 seconds. As oxybuprocaine hydrochloride is an ester that may rarely cause any allergic reaction. Undertaking MRI is safe and causes no pain but having to lie still for about 20 minutes might cause some discomfort or pain, particularly in the case of a recent injury. Although benign, risks of MRI include magnetic/quench hazard and claustrophobia. People suffering from claustrophobia or with a metallic implant in the body were excluded from having an MRI. Having a blood sample taken may cause some discomfort. Rarely, there could be bruising or a minor infection. If this happens, it can be easily treated.

The risk of malfunction of the CCM device was low. We have an active maintenance license with the supplier.

Chapter 3: Prevalence and management of diabetic neuropathy in secondary care in Qatar

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3.1 Abstract

Introduction: Diabetic peripheral neuropathy (DPN) is a 'Cinderella' complication, particularly in the Middle East. A high prevalence of undiagnosed DPN and those at risk of diabetic foot ulceration (DFU) is a major concern.

Objectives: We have determined the prevalence of DPN and its risk factors, DFU and those at risk of (DFU) in patients with T2D in secondary care in Qatar.

Methods: Adults with T2D were randomly selected from the two National Diabetes Centers in Qatar. DPN was defined by the presence of neuropathic symptoms and a vibration perception threshold (VPT)≥15V. Participants with a VPT≥25V were categorized as high risk for DFU. Painful DPN was defined by a DN4 score ≥4. Logistic regression analysis was used to identify predictors of DPN.

Results: In 1,082 adults with T2D (age 54 ±11 years, duration of diabetes 10.0 ±7.7 years, 60.6% males) the prevalence of DPN was 23.0% (95% CI: 20.5%-25.5%), of whom 33.7% (95% CI: 27.9%-39.6%) were at high risk of DFU and 6.3% had DFU. 82.0% of the patients with DPN were previously undiagnosed. The prevalence of DPN increased with age and duration of diabetes and was associated with poor glycemic control (HbA1c≥9%) AOR=2.1 (95%CI: 1.3-3.2), hyperlipidemia AOR=2.7 (95%CI: 1.5-5.0) and hypertension AOR=2.0 (95%CI: 1.2-3.4).

Conclusions: Despite, DPN affecting 23% of adults with T2D, 82% had not been previously diagnosed with 1/3 at high risk for DFU. This argues for annual screening and identification of

patients with DPN. Furthermore, we identify hyperglycemia, hyperlipidemia and hypertension as predictors of DPN.

3.2 Introduction

Diabetic peripheral neuropathy (DPN) imposes a significant health and economic burden to both the patient and health care providers (Raghav et al., 2018). DPN leads to painful DPN (pDPN) in 18-65% (Ponirakis et al., 2019b), erectile dysfunction in 53-73% (Kouidrat et al., 2017) and diabetic foot ulcers in 2-17% (Raghav et al., 2018) of patients with Type 2 diabetes (T2D). One in four patients with diabetic foot ulcer are at risk of amputation (Apelqvist and Agardh, 1992). The prevalence of diabetes in Qatar is almost two-fold higher than the global average of 8.3% and is associated with an increasing prevalence of the long-term complications (IDF Middle East and North Africa Region, 2020, , International Diabetes Federation, 2019,) and is associated with an increasing prevalence of the long term complications (Bener and Al-Hamaq, 2016). Estimates of the prevalence of DPN in people with T2D vary from 17-53% in the Middle East and North Africa (MENA) region (AlAyed et al., 2015, Al-Kaabi et al., 2014, Al-Mahroos and Al-Roomi, 2007), 27-32% in Europe (Young et al., 1993, Salvotelli et al., 2015, Cabezas-Cerrato, 1998), 21-45% in the US (Mold et al., 2004, Cheng et al., 2006) and 17-62% in China (Lu et al., 2010, Liu et al., 2010). This high variability may be attributed to the heterogeneity of the populations studied and differing criteria for the diagnosis of DPN.

Screening annually for symptoms and signs of DPN starting at diagnosis of T2D is recommended by the 2017 American Diabetes Association position statement on DPN (Pop-Busui et al., 2017). However, the prevalence of undiagnosed DPN and those at risk of diabetic foot ulceration (DFU) remains alarmingly high (Wang et al., 2011, Herman and Kennedy, 2005), despite the 5-year mortality of people with a diabetic foot ulcer being higher than many common cancers (Moulik et al., 2003, Armstrong et al., 2007). Indeed in Qatar, 25% of patients attending secondary care were being seen for foot problems (Al-Thani et al., 2019).

Given the lack of disease modifying treatments for DPN (Malik, 2016, Malik, 2014), the identification of risk factors for DPN is key in optimizing treatment and delaying the development and progression of DPN (Pop-Busui et al., 2017). Age and duration of diabetes

are established risk factors for DPN (Al-Kaabi et al., 2014, Young et al., 1993, Cabezas-Cerrato, 1998, Al-Mahroos and Al-Roomi, 2007). Whilst, poor glycemic control is associated with DPN (Al-Mahroos and Al-Roomi, 2007, Boru et al., 2004), there are conflicting data on the benefits of improved glycemic control on DPN (Ohkubo et al., 1995, Ismail-Beigi et al., 2010, Pop-Busui et al., 2013, Azad et al., 1999, Gaede et al., 2003). Studies also suggest that modifiable cardiovascular risk factors including hypertension (Mold et al., 2004, Cardoso et al., 2015, Kesavamoorthy et al., 2015, Yang et al., 2015) and hyperlipidemia (Tesfaye et al., 2005, Smith and Singleton, 2013) are associated with DPN and treatment with angiotensin converting enzyme (ACE) inhibitor (Malik et al., 1998, Ruggenenti et al., 2011, Reja et al., 1995) may improve neuropathy and statins (Arya et al., 2018, Hsu et al., 2017) and fibrates (Rajamani et al., 2009) may reduce amputation.

The objectives of this study were to establish the prevalence of DPN and its risk factors, those at risk of DFU and with DFU in a large cohort of randomly selected people with T2D attending the National Diabetes Centers in Qatar.

3.3 Materials and Methods

Subjects aged 18 - 85 years old with T2D were enrolled from the National Diabetes Centers in Hamad General Hospital (HGH) and Al-Wakra Hospital. 1,161 subjects were randomly enrolled between June 2017 and February 2019. Exclusion criteria included type 1 diabetes, other causes of neuropathy including severe vitamin B_{12} deficiency, chronic hypothyroidism and chemotherapy.

This study was approved by the Institutional Review Board (IRB) of Weill Cornell Medicine-Qatar (WCM-Q) and Hamad Medical Corporation (HMC), and all subjects gave informed consent to take part in the study. The research adhered to the tenets of the declaration of Helsinki.

3.3.1 Demographic and metabolic measures

Age, gender, duration of diabetes and body mass index (BMI) were recorded. Ethnicity was categorized as Qatari Arabs, other Arabs, South Asians, and other ethnic groups. The average systolic (SBP) and diastolic blood pressure (DBP) of two readings were obtained from the

subject's left arm while seated with the arm at heart level, using a standard zero mercury sphygmomanometer after 10-15 minutes of rest. A non-fasting blood sample of 10 ml was collected through venepuncture from each subject into EDTA tubes. The samples were kept at room temperature and transported within 2 hours to a central certified laboratory at HGH. Glycated hemoglobin (HbA1c), total cholesterol and triglyceride were measured by an autoanalyzer (Hitachi 747 autoanalyzer, Japan). Poor glycemic control was defined as HbA1c ≥9%. Hypertension was defined according to either an average SBP ≥140 mmHg and/or the use of anti-hypertensive medication, as described in the WHO/ISH Guidelines (Moser, 1999). Hyperlipidemia was defined according to a total cholesterol level ≥6.2 mmol/L and/or triglyceride level of ≥2.3 mmol/L or if the patient was treated with a statin. Obesity was classified according to WHO criteria with a BMI ≥30 Kg/m^2 (Report of a WHO consultation, 2000). Current cigarette smoking was defined as having smoked at least one cigarette every day for ≥1 year preceding the study visit. Physical activity was defined as doing some physical activity including walking for ≥30 minutes/day for at least 3 times a week.

3.3.2 Assessment of diabetic neuropathy and neuropathic complications

DPN was diagnosed clinically based on the presence of one or more neuropathic symptoms and impaired vibration perception in the feet. Neuropathic symptoms included burning pain, painful cold, electric shocks, tingling, pins and needles and numbness. Vibration perception was measured by a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK) on the pulp of the large toe on both feet. The amplitude of the vibration was slowly increased until it was felt by the participant, and the vibration perception threshold (VPT) was recorded. The average value of three VPT measurements was recorded in Volts (V) ranging from 0 - 50V. A VPT ≥15V was defined as impaired vibration perception (Wiles et al., 1991) and a VPT ≥25V as high risk for diabetic foot ulceration (DFU) (Young et al., 1994). Previously diagnosed DPN was self-reported. Painful DPN (pDPN) was diagnosed using the Douleur Neuropathique en 4 (DN4) questionnaire as previously described (Ponirakis et al., 2019b).

3.3.3 Statistical analysis

The estimated minimum sample size was 937 based on the assumption that the prevalence of DPN was around 25% in a population of 5,000 patients with T2D in SHC with ±2.5 % the acceptance absolute deviation of sample rate from population rate and 95% confidence level.

The overall prevalence of DPN and those at high risk of DFU were computed along with their 95% confidence intervals. Prevalence of DPN across different demographic and health factors as categorical variables was summarized using frequency distributions. Continuous variables were summarized using means and standard deviations. Variables were compared between patients with and without DPN using a Chi-squared test of independence.

Binary logistic regression analysis was performed with age, duration of diabetes, gender, poor glycemic control, obesity, hyperlipidemia, hypertension, physical activity, smoking and ethnic groups as independent variables, and DPN as the dependent variable. Collinearity was tested to minimize its potential effect by selecting variables whose correlation coefficients was <0.7. The multiple logistic regression model included all independent variables associated with DPN with a P value of ≤0.05 at the bivariate level. Adjusted odds ratios, their corresponding 95% confidence intervals (CI) and P value are presented.

All analyses were performed using IBM-SPSS (version 23; SPSS Inc, Armonk NY). A two-tailed P value of ≤0.05 was considered significant.

3.4 Results

3.4.1 Prevalence of DPN and those at risk of DFU

1,082 subjects with T2D (60.6% male) were recruited. We excluded 75 subjects with T1D and 4 subjects with T2D <20 years old. The mean age and duration of diabetes were 54.3 ±11.4 years and 10.0 ±7.7 years, respectively.

The prevalence of DPN was 23.0% (n=249/1,082) (95% CI: 20.5%-25.5%) of whom 33.7% (n=84/249) (95% CI: 27.9%-39.6%) were at high risk of DFU (VPT≥25V) and 6.3% (n=15/237) had diabetic foot ulcers (Table 3.1). Impaired vibration perception in the feet (VPT≥15V) was detected in all subjects with clinical DPN but was also present in 7.2% (n=60/833) of subjects without DPN. A high risk of DFU was detected in 2.2% (n=18/833) of subjects without DPN. Foot ulcers were observed in more subjects with DPN compared to subjects without DPN

(6.3% vs 2.1%, P=0.001) and in more subjects at high risk of DFU compared to subjects at low risk of DFU (11.1% vs 2.2%, P<0.0001). Painful diabetic neuropathy (DN4 score≥4) was present in 24.4% (n=203/833) of subjects without DPN and in 78.7% (n=196/249) of subjects with DPN. 82.0% (n=201/249) of patients with DPN were previously undiagnosed, even though 62.7% of them were aware that they had foot numbness and 7.6% were at high risk of DFU. In those with DPN, 20.5% (n=51/249) were unaware they had impaired vibration perception and 4.8% (n=12/249) were unaware they were at high risk of DFU. The mean total cholesterol (4.3±1.1 vs 4.5±1.2, P<0.01) and low-density lipoprotein (LDL) (2.4±0.9 vs 2.6±0.9, P=0.01) in patients with DPN were significantly lower compared to patients without DPN. Triglycerides (1.7±0.9 vs 1.8±1.3, P=0.14) and high-density lipoprotein (HDL) (1.1±0.4 vs 1.1±0.3, P=0.89) were comparable between patients with and without DPN.

3.4.2 Prevalence of diabetic neuropathy in relation to clinical and demographic factors (Table 3.2)

The prevalence of DPN was lower in those with increasing physical activity (P=0.004, Cramer's V = 0.13) and higher with increasing age (P<0.0001, Cramer's V = 0.21), duration of diabetes (P<0.0001, Cramer's V = 0.29), poor glycemic control (P<0.0001, Cramer's V = 0.08), hyperlipidemia (P<0.0001, Cramer's V = 0.85) and hypertension (P<0.0001, Cramer's V = 0.17) and was comparable between genders. The prevalence of DPN was significantly higher in Qatari Arabs (29.5%) compared to South Asians (17.1%) (P=0.001).

Table 3.1. Prevalence of diabetic neuropathy, impaired vibration perception, high risk of diabetic foot ulcers, painful diabetic neuropathy, and prevalence of patients who are undiagnosed or unaware of diabetic neuropathy.

	Diabetic neuropathy			
	No		Yes	
n %	833	77.0%	249	23.0%
Impaired vibration perception (VPT ≥15V)	60/833	7.2%	249/249	100.0%
High risk of diabetic foot ulceration (VPT ≥25V)	18/833	2.2%	84/249	33.7%
Diabetic foot ulcers	17/809	2.1%	15/237	6.3%
Painful diabetic neuropathy (DN4 ≥4)	203/833	24.4%	196/249	78.7%
Undiagnosed with diabetic neuropathy			201/249	82.0%
Undiagnosed with diabetic neuropathy but aware of foot numbness			156/249	62.7%
Undiagnosed with diabetic neuropathy but at high risk of foot ulceration			19/249	7.6%
Unaware of impaired vibration perception (VPT ≥15V)			51/249	20.5%
Unaware of impaired vibration perception but at high risk of foot ulceration			12/249	4.8%

Table 3.2. Prevalence of diabetic neuropathy in relation to clinical and demographic factors.

		Total		Diabetic neuropathy				
				No		Yes		P value
n (%)		1082	100.0%	833	77.0%	249	23.0%	N/A
Gender	Male	651	60.6%	496	76.2%	155	23.8%	NS
	Female	424	39.4%	333	78.5%	91	21.5%	
Age	20-50 years	440	41.4%	392	89.1%	48	10.9%	<0.0001
	51-60 years	375	35.3%	269	71.7%	106	28.3%	
	>60 years	247	23.3%	156	63.2%	91	36.8%	
Duration of diabetes	≤10 years	682	63.7%	588	86.2%	94	13.8%	<0.0001
	11-20 years	302	28.2%	201	66.6%	101	33.4%	
	>20 years	86	8.0%	37	43.0%	49	57.0%	
Poor glycemic control	No	695	70.8%	562	80.9%	133	19.1%	<0.0001
	Yes	287	29.2%	197	68.6%	90	31.4%	
Obesity	No	442	46.6%	350	79.2%	92	20.8%	NS
	Yes	507	53.4%	383	75.5%	124	24.5%	
Hyperlipidemia	No	236	24.7%	209	88.6%	27	11.4%	<0.0001
	Yes	721	75.3%	526	73.0%	195	27.0%	
Hypertension	No	366	35.5%	318	86.9%	48	13.1%	<0.0001
	Yes	664	64.4%	472	71.0%	193	29.0%	
Physical activity	Yes	321	38.1%	268	83.5%	53	16.5%	0.004
	No	522	61.9%	389	74.5%	133	25.5%	
Smoking	No	742	82.6%	572	77.1%	170	22.9%	NS
	Yes	156	17.4%	128	82.1%	28	17.9%	
Ethnic groups	Qatari Arabs	322	30.0%	227	70.5%	95	29.5%	0.001
	Other Arabs	300	28.0%	233	77.7%	67	22.3%	
	South Asians	397	37.0%	329	82.9%	68	17.1%	
	Others	54	5.0%	38	70.4%	16	29.6%	

Variables were summarized in frequency distribution and compared using χ^2.

3.4.3 Diabetic neuropathy risk factors

The results of binary logistic regression used to explore the odds of developing DPN in relation to age, duration of diabetes, poor glycemic control, hyperlipidemia, hypertension, physical activity and ethnic groups are shown in Table 3.3. Obesity and smoking were not associated with DPN (P=0.2). The odds of developing DPN were 2.5 (95% CI 1.4 – 4.3) times greater among subjects aged 51-60 years (P=0.001) and 3.1 (95% CI 1.7 – 5.7) times greater among subjects aged >60 years compared to subjects aged 20-50 years (P<0.0001). The odds increased from 2.2 (95% CI 1.4 – 3.4) times greater with 11-20 years of diabetes (P=0.001) to 7.2 (95% CI 3.8 – 13.9) times greater with >20 years of diabetes (P<0.0001) compared to those with ≤10 years of diabetes. The odds of developing DPN were 2.1 (95% CI 1.3 – 3.2) times greater with poor glycemic control (P=0.001), 2.7 (95% CI 1.5 – 5.0) times greater with hyperlipidemia (P=0.002) and 2.0 (95% CI 1.2 – 3.4) times greater with hypertension (P=0.01) compared to subjects with HbA1c <9%, without hyperlipidemia and without hypertension. Physical activity was associated with a reduced prevalence of DPN (odds ratio 0.6; 95% CI: 0.4 to 0.8; P=0.002), but after controlling for other significant predictors of DPN, physical activity had no impact on DPN (adjusted odds ratio 0.9; 95% CI: 0.6 to 1.4; P>0.05).

The odds of developing DPN in Qatari Arabs was 1.4 times greater compared to other Arabs (P=0.04) and 2.0 times greater compared to South Asians (P<0.0001). However, these associations with DPN were lost after controlling for other significant predictors of DPN. When comparing the prevalence of risk factors across the ethnic groups, there were more Qataris aged ≥60 years (37.5% vs 24.4% and 11.0%, P<0.0001) and less Qataris aged 20-50 years (30.0% vs 43.5% and 48.7%, P<0.0001) compared to other Arabs and South Asians, respectively. There were also more Qataris with 11-20 years of diabetes compared to other Arabs and South Asians (40.1% vs 23.4% and 22.0%, P<0.0001) whilst there were less Qataris with ≤10 years of diabetes (47.5% vs 69.9% and 72.5%, P<0.0001). The prevalence of hypertension in Qataris was higher compared to other Arabs (72.5% vs 56.4%, P=0.001) but comparable with South Asians (64.0%).

Table 3.3. Predictors for diabetic neuropathy using multiple logistic regression analysis.

Independent variables		AOR	95% CI	P value
Age	20-50 years	1		
	51-60 years	2.5	1.4 – 4.3	0.001
	>60 years	3.1	1.7 – 5.7	<0.0001
Duration of diabetes	≤10 years	1		
	11-20 years	2.2	1.4 – 3.4	0.001
	>20 years	7.2	3.8 – 13.9	<0.0001
Poor glycemic control		2.1	1.3 - 3.2	0.001
Hyperlipidemia		2.7	1.5 - 5.0	0.002
Hypertension		2.0	1.2 - 3.4	0.01
Physical activity		0.9	0.6 - 1.4	NS
Ethnic groups	Qatari Arabs	1		
	Other Arabs	1.0	0.6 - 1.6	NS
	South Asians	0.7	0.4 - 1.1	NS
	Others	1.4	0.5 – 3.9	NS

Outcome variable: diabetic neuropathy. Independent variables: Age, duration of diabetes, poor glycemic control, hyperlipidemia, hypertension, physical activity and ethnic groups were considered in the fitted model with a P value ≤0.05. AOR=Adjusted odd ratio; CI= confidence interval.

3.5 Discussion

In adults with T2D attending secondary care in Qatar the prevalence of DPN was 23%, of whom one-third were at high risk of DFU, and 6% had diabetic foot ulcers. However, 82% of patients with DPN had not been previously diagnosed, even though 63% were aware they had foot numbness. Age, duration of diabetes, poor glycemic control, hyperlipidemia and hypertension are risk factors for DPN in this population.

The prevalence of DPN varies in different countries and clinical settings. In a large clinic based study of 6487 patients in the UK, the prevalence of DPN was 32.1% in patients with T2D and increased with increasing age and duration of diabetes (Young et al., 1993). It has been reported to be as high as 45% in the US (Mold et al., 2004) and 62%, in China (Lu et al., 2010), but their mean age of 73 and 66 years, respectively was much higher than our cohort aged 54 years. The higher prevalence of DPN in Bahrain (37%)(Al-Mahroos and Al-Roomi, 2007) and Turkey (60%)(Boru et al., 2004) compared to Qatar (23%) may be attributed to poorer glycemic control as the proportion with a HbA1c ≥9% in Bahrain (65%) and Turkey (79%) was much higher compared to our cohort in Qatar (29%).

We show an alarmingly high prevalence of undiagnosed DPN in 82% of patients attending secondary care in Qatar. Indeed, Wang et. al. (Wang et al., 2011) have also previously

reported that 79% of patients with T2D have undiagnosed DPN and Herman et al. (Herman and Kennedy, 2005) reported that 62% of patients with T2D have undiagnosed DPN in the US had not been previously diagnosed. Wang et. al. (Wang et al., 2011) did not specify what diagnostic criteria for DPN they used for the study. Herman et al. (Herman and Kennedy, 2005) used monofilament test to screen for DPN, which only detects advanced large fiber neuropathy. This may explain why the percentage of underdiagnosed DPN is lower compared to our study. The high prevalence of undiagnosed DPN in secondary care in Qatar can be attributed to the lack of annual screening for DPN and use of the 10-g monofilament which will identify only those with advanced neuropathy (Pop-Busui et al., 2017). The prevalence of DFU in Qatar was comparable to the global prevalence of 6.3% (Zhang et al., 2017b).

The early diagnosis and treatment of DPN is key in preventing DFU and amputation (Pop-Busui et al., 2017). Indeed, in line with previous studies (Al-Mahroos and Al-Roomi, 2007, Boru et al., 2004) we show that poor glycemic control is an independent risk factor for DPN. The Kumamoto trial (Ohkubo et al., 1995) reported that tight glucose control prevents progression of DPN and the ACCORD (Ismail-Beigi et al., 2010) and the BARI 2D (Pop-Busui et al., 2013) trials reported a reduced incidence of DPN with better glycemic control. However, the UKPDS (UK Prospective Diabetes Study (UKPDS) Group, 1998), VA-CSDM (Azad et al., 1999) and Steno-2 trial (Gaede et al., 2003) have shown a limited effect of intensive glucose control on DPN. This study shows an association of DPN with hyperlipidemia and hypertension, which is consistent with previous studies showing that DPN is associated with hypertension (Mold et al., 2004, Cardoso et al., 2015, Kesavamoorthy et al., 2015, Yang et al., 2015), hyperlipidemia (Tesfaye et al., 2005, Smith and Singleton, 2013), BMI (Al-Mahroos and Al-Roomi, 2007, Salvotelli et al., 2015, Mold et al., 2004), cigarette smoking (Al-Mahroos and Al-Roomi, 2007, Tesfaye et al., 2005) and physical activity (Al-Kaabi et al., 2014). Indeed, treatment with angiotensin converting enzyme (ACE) inhibitors (Malik et al., 1998, Ruggenenti et al., 2011, Reja et al., 1995) and statins (Davis et al., 2008, Villegas-Rivera et al., 2015) may slow the progression of DPN. We also show a relationship between reduced physical activity and the prevalence of DPN, which is consistent with a study showing that diet and exercise can improve neuropathy in subjects with impaired glucose tolerance (Smith et al., 2006). Previously, we reported that South Asians, have a lower prevalence of DPN compared to Caucasians (Abbott et al., 2010), particularly small fiber neuropathy (Fadavi et al., 2018). This

study shows that South Asians have a lower prevalence of DPN compared to Qatari Arabs but the association between ethnic groups and DPN was lost after controlling for significant predictors of DPN including, age, duration of diabetes, poor glycemic control, hyperlipidemia, hypertension and physical activity as these factors differed in the different ethnicities.

There are several limitations of this study including the diagnosis of DPN which was based on symptoms and assessment of VPT as reflected by the finding that 7.2% of participants without clinical DPN had an abnormal VPT. This may reflect issues with the reliability and validity of establishing a vibration perception threshold value which like all psychophysical tests relies on standardization and the participants concentration and ability to detect a sensation. We acknowledge that recruiting patients with T2D from secondary health care centers and not primary care centers limits the generalizability of the results to all people with T2D in Qatar. However, the recruited participants were of diverse backgrounds. Whilst we show associations between risk factors and DPN, the cross-sectional design of this study limits the predictive validity of these risk factors.

In conclusion, although the prevalence of DPN was relatively low compared to previous studies from the Middle East region, alarmingly 82% were undiagnosed and one-third of patients with DPN were at high risk of DFU, highlighting the need for screening for DPN. This study argues for annual screening and identification of patients with DPN for more aggressive treatment of hyperglycemia, hyperlipidemia and hypertension..

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Chapter 4: Prevalence and risk factors for painful diabetic neuropathy in secondary healthcare in Qatar

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4.1 Abstract

Introduction: Painful diabetic peripheral neuropathy (pDPN) has a significant impact on the patient's quality of life. The prevalence of pDPN in the Middle East and North Africa (MENA) region has been reported to be almost double that of populations in the UK.

Objectives: We sought to determine the prevalence of pDPN and its associated factors in T2D patients attending secondary care in Qatar.

Methods: This is a cross-sectional study of 1095 participants with T2D attending Qatar's two national diabetes centers. PDPN and impaired vibration perception on the pulp of the large toes were assessed using the DN4 questionnaire with a cut-off ≥4 and the Neurothesiometer with a cut-off ≥15V, respectively.

Results: The prevalence of pDPN was 34.5% (95% CI: 31.7%-37.3%), but 80% of these patients had not previously been diagnosed or treated for this condition. Arabs had a higher prevalence of pDPN compared to South Asians (P<0.05). PDPN was associated with impaired vibration perception AOR=4.42 (95%CI: 2.92-6.70), smoking AOR=2.43 (95%CI: 1.43-4.15), obesity AOR=1.74 (95%CI: 1.13-2.66), being female AOR=1.65 (95%CI: 1.03-2.64) and duration of diabetes AOR=1.08 (95%CI: 1.05-1.11). Age, poor glycemic control, hypertension, physical activity and proteinuria showed no association with pDPN.

Conclusions: PDPN occurs in 1/3 of T2D patients attending secondary care in Qatar, but the majority have not been diagnosed. Arabs are at higher risk for pDPN. Impaired vibration perception, obesity and smoking are associated with pDPN in Qatar.

4.2 Introduction

Painful diabetic peripheral neuropathy (pDPN) has a significant impact on the patient's quality of life (Van Acker et al., 2009, Bohlega et al., 2010, daCosta DiBonaventura et al., 2011) as it is accompanied by depression, anxiety and sleep disturbance (Bohlega et al., 2010). Estimates of the prevalence of pDPN in patients with T2D vary and range from 17.9%-65.3% (Van Acker et al., 2009, Abbott et al., 2011, Jambart et al., 2011, Halawa et al., 2010). In a large population-based study (n=15,692) from the UK (Abbott et al., 2011), we previously showed that pDPN occurred in 21.5% of patients with T2D and was more common in South Asians. In the Middle East and North Africa (MENA) region, Jambart *et al.* (Jambart et al., 2011) reported a much higher prevalence of pDPN of 61.3% in Egypt, 57.5% in Jordan, 53.9% in Lebanon and 37.1% in the United Arab Emirates.

Despite having a serious impact on the patient's quality of life, pDPN is underdiagnosed and undertreated (Ziegler et al., 2018, Daousi et al., 2004). Patients with painful symptoms are often unaware that the pain is related to diabetes and do not report it to their clinician (Daousi et al., 2004, Eichholz et al., 2017). Screening patients at high risk for pDPN should allow timely identification and treatment. Previous studies have shown that older age, a longer duration of diabetes, being female and the presence of diabetic peripheral neuropathy (DPN) increases the risk for pDPN (Davies et al., 2006, Jambart et al., 2011, Halawa et al., 2010, Van Acker et al., 2009, Abbott et al., 2011, Jacovides et al., 2014). Additionally, obesity (Jambart et al., 2011, Van Acker et al., 2009, Ziegler et al., 2018, Aslam et al., 2015), low physical activity (Ziegler et al., 2009, Smith et al., 2006), smoking (Abbott et al., 2011, Aslam et al., 2015), poor glycemic control (Harris et al., 1993, Smith and Singleton, 2008), low HDL cholesterol (Van Acker et al., 2009), raised LDL cholesterol, triglycerides and creatinine (Ziegler et al., 2009), are also independent risk factors of pDPN.

The aim of this study was to establish the prevalence of pDPN in patients with T2D in secondary care in Qatar and explore the association with ethnicity and risk factors for this condition. We have undertaken a large cross-sectional cohort study using DN4, a validated and highly sensitive and specific questionnaire for the diagnosis of pDPN (Spallone et al., 2012, Terkawi et al., 2017).

4.3 Materials and Methods

This is a cross-sectional cohort study. Patients with diabetes aged 18 years and above were recruited from the two National Diabetes & Endocrine Centers in Qatar, Hamad General Hospital and Al-Wakra Hospital. Participating clinicians reported on all patients satisfying the inclusion criteria, examined between March 2017 to March 2018. No refusals were recorded as the procedure was quick, simple and potentially valuable to the patient health. Participants with other causes of neuropathy including vitamin B_{12} deficiency, hypothyroidism, HIV infection, leprosy, hepatitis C and chemotherapy were excluded from the study. We enrolled 1,163 individuals and after excluding 66 patients with T1D and 2 patients who did not complete the assessments were left with a sample size of 1,095.

This study was approved by the Institutional Review Board (IRB) of WCM-Q and HMC and all participants gave informed consent to take part in the study. The research adhered to the tenets of the declaration of Helsinki.

4.3.1 Demographic and metabolic measures

Age, gender, duration of diabetes, height, weight and BMI were recorded. Ethnicity was categorized as Qatari Arabs, other Arabs, South Asians, and other ethnic groups. The average of two readings of the systolic (SBP) and diastolic (DBP) blood pressure taken from the subject's left arm while seated with his/her arm at heart level, using a standard zero mercury sphygmomanometer after 10-15 minutes of rest was obtained. A non-fasting blood sample of 10 ml was collected through venepuncture from each participant into vacutainer tubes containing EDTA. The samples were kept at room temperature and transported within 2 hours to a central certified laboratory at Hamad General Hospital, HMC, Doha, Qatar. Glycated hemoglobin (HbA1c), total cholesterol, HDL, LDL and triglycerides were measured by an autoanalyzer (Hitachi 747 autoanalyzer, Japan). Urinary albumin and creatinine levels were assessed on a random spot urine sample to evaluate the albumin-to-creatinine ratio (ACR). Patients with an HbA1c ≥9% were considered to be poorly controlled. Hypertension was defined according to either an average systolic blood pressure (SBP) ≥ 140 mmHg and/or the use of antihypertensive medication, as described in the WHO/ISH Guidelines (Moser, 1999). Current cigarette smoking was defined as having smoked at least one cigarette every day for

30 days preceding the study visit. Physical activity was defined as doing physical activity including walking for 30 minutes or more in a day for at least 3 times a week. Obesity was classified according to WHO criteria (Report of a WHO consultation, 2000) with a BMI ≥30 Kg/m^2. Proteinuria was defined as an ACR >30mg/g.

4.3.2 Painful diabetic peripheral neuropathy assessment

The Douleur Neuropathique en 4 (DN4) questionnaire has been validated for painful diabetic peripheral neuropathy (pDPN) (Spallone et al., 2012) and can distinguish between nociceptive and neuropathic pain (Harifi et al., 2011). It consists of 10 questions: 7 questions relating to the pain description (burning, painful cold, electric shocks) and associated abnormal sensations (tingling, pins and needles, numbness, itching) and the other 3 questions relate to a neurological examination in the painful area (hypoesthesia to touch and prick using disposable examination pins and allodynia to brushing). The scoring is based on a yes (1 point) or no (0 point) answer and each question is equally weighted. A score ≥4 has a high sensitivity (80%) and specificity (92%) for pDPN (Spallone et al., 2012). The questionnaire was administered by the investigator spoken in either English or Arabic. Previously diagnosed pDPN was self-reported. Medications for painful neuropathy were recorded.

4.3.3 Impaired vibration perception assessment

Vibration perception threshold (VPT) was measured bilaterally on the pulp of the large toe using a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK). The strength of the vibration stimulus was gradually increased from null intensity to a value in voltage at which vibration was first detected by the participant. The test was repeated three times and the average value was recorded. The range for VPT readings is 1 to 50V. Impaired vibration perception was defined on a mean VPT ≥15V (Wiles et al., 1991, Garrow and Boulton, 2006).

4.3.4 Statistical analysis

The estimated minimum sample size was 1027 based on the assumption that the prevalence of pDPN was around 30% in a population of 5,000 patients with T2D in SHC with ±2.5 % the acceptance absolute deviation of sample rate from population rate and 95% confidence level.

Patients' demographic and clinical characteristics were summarized using means and standard deviations for numeric variables and frequency distribution for categorical variables. Continuous parametric variables were compared between patients with and without pDPN using unpaired t-test or non-parametric variables when the distribution had skewness of < -1 or > 1 with Mann-Whitney test. Categorical variables were compared using Chi-squared test or Fisher's exact test when expected cell counts fell below 5.

Binary and multiple logistic regression analysis was performed with age, duration of diabetes, diabetic neuropathy, gender, poor glycemic control, hypertension, obesity, physical activity, smoking, proteinuria and ethnic groups as independent variables, and pDPN as the dependent variable. The multiple logistic regression model included all variables with p-value of 0.10 or less at the bivariate level. Adjusted odds ratios and their corresponding 95% confidence intervals are presented.

Demographic and clinical characteristics of the patients were compared between the different ethnic groups using the chi-square test for categorical variables such as hypertension and one-way ANOVA for numeric variables such as age. Multiple comparisons when needed were done using the Bonferroni's method.

All analyses were performed using IBM-SPSS (version 23; SPSS Inc, Armonk NY). A two-tailed P value of ≤0.05 was considered significant.

4.4 Results

4.4.1 Prevalence of pDPN

The cohort (n=1095) was aged 20 to 86 years (mean±SD, 54.3±11.4), 60.6% were male. The clinical and demographic characteristics of T2D subjects with and without painful diabetic peripheral neuropathy (pDPN) are compared in Table 4.1. The prevalence of pDPN was 34.5% (95% CI: 31.7%-37.3%). 80.2% of the subjects with pDPN had not been previously diagnosed with this condition and 86.0% had not been treated.

Table 4.1. Demographic characteristics of adults with T2D stratified by pDPN status. Patients' demographic and clinical characteristics summarized using means and standard deviations for numeric variables and frequency distribution for categorical variables. Continuous parametric and non-parametric variables were compared using unpaired t-test and (*) Mann-Whitney test, respectively. Categorical variables were compared using χ^2.

		Painful diabetic neuropathy				
		No		Yes		P value
n (%)		717	(65.5)	378	(34.5)	N/A
Age, years, mean (SD)		52.6	± 11.4	57.5	± 10.7	<0.0001*
Gender, n (%)	Male	453	(68.7)	206	(31.3)	<0.01
	Female	261	(60.7)	169	(39.3)	
Diabetes duration, years, mean (SD)		8.2	± 7.0	13.6	± 7.9	<0.0001*
HbA1c, mean (SD)	%	8.0	± 2.0	8.4	± 2.0	0.02
	mmol/mol	64.9	± 22.3	67.9	± 21.8	0.02
Poor glycemic control	Yes	174	(60.4)	114	(39.6)	<0.05
	No	474	(67.6)	227	(32.4)	
Cholesterol, mmol/l, mean (SD)		4.5	± 1.2	4.4	± 1.1	NS
Triglyceride, mmol/l, mean (SD)		1.9	± 1.3	1.7	± 1.0	NS*
HDL, mmol/l, mean (SD)		1.3	± 0.2	1.1	± 0.0	NS
LDL, mmol/l, mean (SD)		2.6	± 0.0	2.5	± 0.0	NS
Systolic blood pressure, mmHg, mean (SD)		131.1	± 17.7	135.4	± 18.3	<0.001
Diastolic blood pressure, mmHg, mean (SD)		78.5	± 10.5	77.6	± 9.5	NS
Hypertension, n (%)	Yes	371	(61.0)	237	(39.0)	0.001
	No	294	(71.5)	117	(28.5)	
Weight, Kg, mean (SD)		83.4	± 21.4	87.6	± 18.6	<0.0001*
BMI, Kg/m^2, mean (SD)		30.7	± 6.8	32.7	± 7.0	<0.0001
Obesity, n (%), n (%)	Yes	314	(60.2)	208	(39.8)	<0.0001
	No	318	(73.3)	116	(26.7)	
Physical activity, n (%)	Yes	240	(74.5)	82	(25.5)	0.001
	No	330	(63.2)	192	(36.8)	
Smoking, n (%)	Yes	107	(69.0)	48	(31.0)	NS
	No	501	(67.2)	244	(32.8)	
Proteinuria, n (%)	Yes	33	(51.6)	31	(48.4)	<0.01
	No	300	(67.1)	147	(32.9)	
Vibration perception threshold, V, mean (SD)		9.8	± 7.5	17.4	± 10.6	<0.0001
Impaired vibration perception, n (%)	Yes	126	(39.1)	196	(60.9)	<0.0001
	No	586	(76.8)	177	(23.2)	
Previously diagnosed with pDPN, n (%)		28	(4.0)	73	(19.8)	<0.0001
Treated for pDPN, n (%)		22	(3.1)	53	(14.0)	<0.0001
Ethnic groups, n (%)	Qataris	181	(54.7)	150	(45.3)	<0.0001
	Other Arabs	196	(64.3)	109	(35.7)	
	South Asians	299	(74.2)	104	(25.8)	
	Others	41	(73.2)	15	(26.8)	

4.4.2 Factors associated with pDPN

Subjects with pDPN had a higher mean age (P<0.0001, Cohen's d = 0.43), duration of diabetes (P<0.0001, Cohen's d = 7.28), HbA1c (P=0.02, Cohen's d = 1.99), systolic blood pressure (P<0.001, Cohen's d = 0.26), weight (P<0.0001, Cohen's d = 0.21) and BMI (P<0.0001, Cohen's d = 0.31), compared to subjects without pDPN. Vibration perception threshold (VPT) was significantly higher (17.4V vs 9.8V, P<0.0001). Total cholesterol, triglycerides, HDL, LDL and diastolic blood pressure were comparable between the two groups. Subjects with pDPN had a higher percentage of subjects with impaired vibration perception (60.9% vs 23.2%, P<0.0001), a greater proportion of females (39.3% vs 31.3%, P<0.01), poorer glycemic control (39.6% vs 32.4%, P<0.05), more hypertension (39.0% vs 28.5%, P=0.001), greater proportion with proteinuria (48.4% vs 32.9%, P<0.01), more obesity (39.8% vs 26.7%, P<0.0001) and a lower percentage of those undertaking physical activity (25.5% vs 36.8%, P=0.001).

Logistic regression analysis showed that five factors were independently and significantly associated with pDPN (Table 4.2). Impaired vibration perception adjusted odds ratio (AOR)=4.42 (95%CI: 2.92-6.70), smoking AOR=2.43 (95%CI: 1.43-4.15), obesity AOR=1.74 (95%CI: 1.13-2.66), being female AOR=1.65 (95%CI: 1.03-2.64) and duration of diabetes =1.08 (95%CI: 1.05-1.11) were associated with pDPN. Age, poor glycemic control, hypertension, physical activity, proteinuria and ethnicity showed no association with pDPN.

Table 4.2. Logistic regression analysis between painful diabetic peripheral neuropathy and risk factors. Outcome variable: Painful diabetic peripheral neuropathy. Independent variables: Age, duration of diabetes, impaired vibration perception, female, poor glycemic control, hypertension, obesity, physical activity, smoking, proteinuria and ethnic groups were considered in the fitted model with a P value ≤0.05.

	AOR	(95% CI)		P value
Age	1.01	(0.99 -	1.03)	0.28
Duration of diabetes	1.08	(1.05 -	1.11)	<0.0001
impaired vibration perception	4.42	(2.92 -	6.70)	<0.0001
Female	1.65	(1.03 -	2.64)	<0.05
Poor glycemic control	1.40	(0.93 -	2.11)	0.28
Hypertension	1.16	(0.77 -	1.76)	0.64
Obesity	1.74	(1.13 -	2.66)	<0.01
Physical activity	0.83	(0.55 -	1.26)	0.09
Smoking	2.43	(1.43 -	4.15)	0.001
Proteinuria	1.04	(0.51 -	2.16)	0.77
Ethnic groups				
Qataris	1			
Other Arabs	1.05	(0.64 -	1.73)	0.44
South Asians	0.95	(0.57 -	1.59)	0.80
Others	0.81	(0.31 -	2.07)	0.37

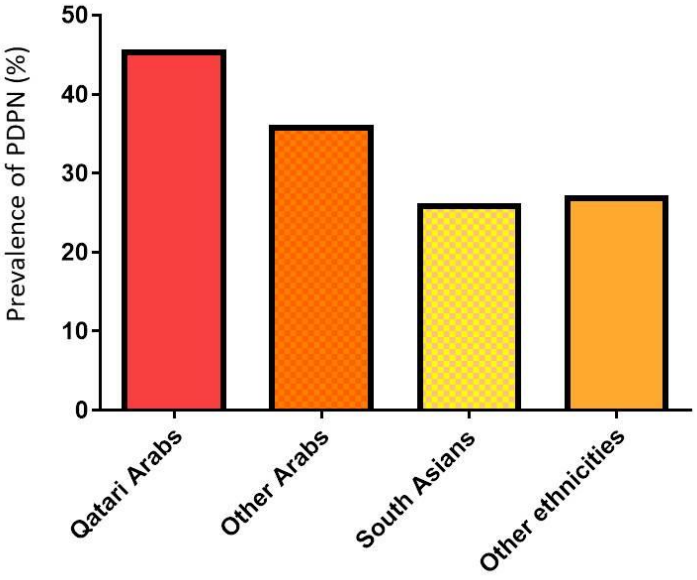
4.4.3 Ethnicity and pDPN

The prevalence of pDPN differed between ethnic groups (Figure 4.1 and Table 4.3). Qataris (45.3%) and other Arabs (35.7%) had a higher prevalence of pDPN compared to South Asians (25.8%). However, the prevalence of impaired vibration perception was comparable between ethnic groups. The prevalence of obesity was comparable between Qataris (66.8%) and other Arabs (70.9%), but significantly higher than in South Asians (34.2%). The percentage of Qataris (20.8%) and other Arabs (35.5%) who undertook physical activity was significantly lower than in South Asians (54.3%). The percentage of Qataris with proteinuria was significantly higher than in South Asians (9.4% vs 3.0%) and comparable with other Arabs and other ethnicities. Qataris were significantly older than other Arabs, South Asians and other ethnicities (58.2 vs 53.8 vs 51.8 and 52.5 years, respectively) and had a significantly longer duration of diabetes (13.4 vs 9.1 vs 8.1 and 9.9 years, respectively). The percentage of Qataris with hypertension was significantly higher than other Arabs (65.3% vs 53.9%). There were significantly less smokers amongst Qataris compared to other Arabs (10.4% vs 23.9%).

Table 4.3. Differences in the prevalence of painful diabetic peripheral neuropathy and other risk factors between different ethnic groups. a,b,c,d within each row, columns with similar letters are not statistically significant and those with different letters are significantly different.

	Qataris	Other Arabs	South Asians	Others
n	331	305	403	56
Painful DPN, n (%)	150 (45.3)[a]	109 (35.7)[a]	104 (25.8)[b]	15 (26.8)[ab]
Age, years, mean (SD)	58.2 (12.0)[a]	53.8 (11.7)[b]	51.8 (9.7)[b]	52.5 (10.5)[b]
Duration of diabetes, years, mean (SD)	13.4 (7.8)[a]	9.1 (7.2)[b]	8.1 (7.0)[b]	9.9 (8.4)[b]
Impaired vibration perception, n (%)	108 (33.0)[a]	91 (30.0)[a]	102 (25.6)[a]	21 (37.5)[a]
Female, n (%)	211 (64.1)[a]	109 (35.9)[b]	89 (22.3)[c]	21 (39.5)[bc]
Poor glycemic control, n (%)	100 (33.8)[a]	86 (31.5)[a]	152 (41.4)[a]	21 (39.6)[a]
Hypertension, n (%)	196 (65.3)[a]	153 (53.9)[b]	229 (59.9)[ab]	30 (56.6)[ab]
Obesity, n (%)	185 (66.8)[ab]	188 (70.9)[b]	125 (34.2)[c]	24 (49.0)[ac]
Physical activity, n (%)	52 (20.8)[a]	87 (35.5)[b]	170 (54.3)[c]	13 (36.1)[abc]
Smoking, n (%)	27 (10.4)[a]	62 (23.9)[b]	57 (16.9)[ab]	9 (20.5)[ab]
Proteinuria, n (%)	31 (9.4)[a]	15 (4.9)[a,b]	12 (3.0)[b]	6 (10.7)[a]

Figure 4.1. Prevalence of painful diabetic peripheral neuropathy between ethnic groups.



4.5 Discussion

This is the first large observational study to establish the prevalence of painful diabetic peripheral neuropathy (pDPN) and its associated factors in secondary care in Qatar. PDPN occurs in approximately one third of patients with T2D, however, alarmingly, 4/5 had not been previously diagnosed or treated. PDPN, a manifestation of small fiber damage (Sorensen

et al., 2006, Vlckova-Moravcova et al., 2008, Quattrini et al., 2007b), occurred in more than one in four patients without impaired vibration perception, and in one in two patients with impaired vibration perception. Impaired vibration perception, obesity and smoking were associated with pDPN. Arabs also have a higher prevalence of pDPN compared to Asians. This may be attributed to the higher percentage of women and obesity, and a lower percentage undertaking physical activity in the Arab population.

The prevalence of pDPN in T2D patients in Qatar was lower than previous studies from the MENA region, even though they also used the Douleur Neuropathique 4 (DN4) pain questionnaire and showed that the prevalence of pDPN was 65.3% in Saudi Arabia (Halawa et al., 2010), 61.3% in Egypt (Jambart et al., 2011), 57.5% in Jordan, 53.9% in Lebanon and 37.1% in United Arab Emirates and Kuwait. This difference could be attributed to different populations and control of various risk factors, although age, duration of diabetes and the percentage of those with obesity were comparable to this study. However, the percentage of those with poor glycemic control in Saudi Arabia was higher compared to the current study (59.5% vs 39.6%) (Akbar et al., 2000). Poor glycemic control is common in the Middle East (Akbar et al., 2000, Youssef et al., 2006, Uddin et al., 2001, Habib and Aslam, 2003) and has been reported to be a significant risk factor for both DPN and pDPN (Harris et al., 1993, Smith and Singleton, 2008). In the UK, the prevalence of pDPN in T2D patients is lower (21.5% - 26.4%) than in Qatar (Abbott et al., 2011, Davies et al., 2006) and may be attributed to a lower HbA1c (7.26% vs 8.14%) and shorter duration of diabetes (4-8 years vs 10.1 years). One of the earlier UK studies (Davies et al., 2006) was conducted in patients with T1D and T2D in primary care and the prevalence of pDPN is known to be lower in primary care (Aslam et al., 2015) and in T1D patients (Abbott et al., 2011, Van Acker et al., 2009, Ziegler et al., 2018).

The physical quality of life of patients with pDPN decreases at a significantly faster rate over 3 years compared to T2D patients without pDPN (daCosta DiBonaventura et al., 2011). Patients with pDPN are also at high risk for depression, anxiety and sleep disturbance (Bohlega et al., 2010). However, the under-diagnosis and treatment of pDPN continues to pose a considerable problem for patients. Other studies have also reported that a large proportion of patients with pDPN were not diagnosed, 61.5% in Germany (Ziegler et al., 2018) and 12.5% in the UK (Daousi et al., 2004). Major hurdles limiting the diagnosis of pDPN are

that patients with painful symptoms do not attribute them to diabetes and fail to report them to their physician (Daousi et al., 2004, Eichholz et al., 2017) and of course screening is not currently advocated for pDPN, only for those at high risk of foot ulceration (Pop-Busui et al., 2017). Given that we have identified age, duration of diabetes and the presence of impaired vibration perception as major determinants for pDPN (Davies et al., 2006, Jambart et al., 2011, Halawa et al., 2010, Van Acker et al., 2009) one could advocate screening for pDPN in at least diabetic patients who are older, have a longer duration of diabetes and impaired vibration perception. Furthermore, we have identified that obesity is associated with pDPN, which has also been reported in some (Jambart et al., 2011, Van Acker et al., 2009, Ziegler et al., 2018, Aslam et al., 2015), but not other studies (Halawa et al., 2010, Jacovides et al., 2014). Low physical activity has been reported as a risk factor (Ziegler et al., 2009, Smith et al., 2006), but in this study we show no association after adjusting for other risk factors. Smoking has also been associated with pDPN in some (Abbott et al., 2011, Aslam et al., 2015) but not other studies (Halawa et al., 2010, Jambart et al., 2011, Jacovides et al., 2014, Abbott et al., 2011, Van Acker et al., 2009). Improved glycemic control reduces the development and progression of DPN in T1D (Klein et al., 1996), but has shown limited benefit in T2D (Callaghan et al., 2012). Low HDL cholesterol raised LDL cholesterol and triglycerides have been independently associated with pDPN (Van Acker et al., 2009). Creatinine is associated with pDPN, whilst albuminuria (Ziegler et al., 2009) and proteinuria have no association. A previous study of subjects with pre-diabetes showed that lifestyle intervention reduced neuropathic symptoms and improved small fiber function and structure (Smith et al., 2006).

The prevalence of painful neuropathic symptoms (Abbott et al., 2011) and pDPN (Eichholz et al., 2017) differs between ethnic groups. In our previous study in the UK (Abbott et al., 2011), we showed that South Asians were 50% more likely to have painful neuropathic symptoms compared to Europeans and Afro-Caribbean's, after adjusting for age and duration of diabetes. However, in the present study, South Asians had a lower prevalence of pDPN compared to Qatari Arabs and other Arabs, which may be attributed to a lower proportion with obesity, less women and higher physical activity in this group. Indeed, this and other studies (Jambart et al., 2011, Abbott et al., 2011) have shown that women have a 50-65% increase in the odds for pDPN. The ethnic difference may also reflect genetic differences in

the prevalence of abnormalities in voltage gated channels on nociceptors in different ethnic groups (Wadhawan et al., 2017, Blesneac et al., 2018).

We recognize that recruiting patients with diabetes from secondary health care centers and not primary care centers as a major limitation of this study and limits the generalizability of the results to all people with diabetes in Qatar. However, those two hospitals are the only National Diabetes & Endocrine centers in Qatar and the recruited participants were of diverse backgrounds. The cross-sectional design of this study also limits the interpretation of cause and effect in relation to risk factors. The strength of this study is the large sample size and the inclusion of a wide range of risk factors to identify those associated independently with pDPN. Furthermore, pDPN was diagnosed using the DN4 questionnaire, which has been validated in Arabic (Harifi et al., 2011) and used in other studies in the MENA region to establish the prevalence of pDPN (Jambart et al., 2011, Halawa et al., 2010).

In conclusion, one in three patients with T2D attending secondary care in Qatar have pDPN. It remains a neglected complication of diabetes as ~80% of patients were not diagnosed or treated for this condition. Impaired vibration perception, obesity and smoking are associated with pDPN, suggesting that patients with these risk factors should be screened for pDPN and treated for relief of symptoms and with life-style interventions to limit progression.

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Chapter 5: Prevalence and risk factors for diabetic neuropathy and painful diabetic neuropathy in primary and secondary health care in Qatar

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5.1 Abstract

Introduction: Diabetic peripheral neuropathy (DPN) can result in painful DPN (pDPN) and diabetic foot ulceration (DFU).

Objectives: This study determined the prevalence and risk factors for DPN and pDPN in patients with type 2 diabetes (T2D) in primary health care (PHC) and secondary health care (SHC) in Qatar.

Methods: This is a cross-sectional multi-center study. Adults with T2D aged 18-85 years old were randomly enrolled from four PHC centers and two Diabetes Centers in SHC in Qatar. Subjects underwent assessment of clinical and metabolic parameters, DPN and pDPN.

Results: 1,386 subjects with T2D were recruited, with 297 from PHC and 1,089 from SHC. The prevalence of DPN (14.8% vs 23.9%, P=0.001) and pDPN (18.1% vs 37.5%, P<0.0001) was significantly lower in PHC compared to SHC and those with DPN at high risk for DFU (31.8% vs 40.0%, P=0.3) was comparable. The prevalence of undiagnosed DPN (79.5% vs 82.3%, P=0.66) was comparably high but undiagnosed pDPN (24.1% vs 71.5%, P<0.0001) was lower in PHC compared to SHC. The odds of DPN and pDPN increased with age and diabetes duration and DPN increased with poor glycemic control, hyperlipidemia and hypertension, whilst pDPN increased with obesity and reduced physical activity.

Conclusions: The prevalence of DPN and pDPN in T2D is lower in PHC compared to SHC and is attributed to overall better control of risk factors and referral bias due to patients with

poorly managed complications being referred to SHC. However, approximately 80% of patients had not been previously diagnosed with DPN in PHC and SHC. Further, we identify a number of modifiable risk factors for PDN and pDPN.

5.2 Introduction

Diabetic peripheral neuropathy (DPN) is the most common complication of diabetes and yet often remains undiagnosed (Pop-Busui et al., 2017). Late diagnosis can lead to significant morbidity in the form of painful DPN (pDPN) (Ponirakis et al., 2019b), erectile dysfunction (Kouidrat et al., 2017), diabetic foot ulceration (DFU) (Raghav et al., 2018) and amputation (Apelqvist and Agardh, 1992), as well as increased mortality (Azmi et al., 2019a).

Early diagnosis and management of DPN may limit or reduce disease progression (Pop-Busui et al., 2017). However, screening for DPN and pDPN is inadequate (Herman and Kennedy, 2005, Wang et al., 2011, Ponirakis et al., 2020b). The prevalence of DPN and pDPN have been shown to range from 2.4-24.1% (Cabezas-Cerrato, 1998, Kostev et al., 2014) and 16-19% (Davies et al., 2006, Daousi et al., 2004) in primary care and 32.1% (Young et al., 1993) and 21.0% (Abbott et al., 2011) in secondary care in patients with type 2 diabetes (T2D), respectively. This wide range has been attributed to differing populations and methods used to identify DPN and pDPN. We have recently reported that approximately 80% of patients with DPN (Ponirakis et al., 2020b) and pDPN (Ponirakis et al., 2019b) have not previously been diagnosed in hospital clinics in secondary health care (SHC) in Qatar, which may lead to late presentation with DFU. Indeed, in Qatar it has been reported that 25% of patients with diabetes in SHC have foot problems (Al-Thani et al., 2019). This has serious consequences given that one in four patients with DFU are at risk of amputation (Apelqvist and Agardh, 1992) and the 5-year mortality of people with a DFU is higher than many common cancers (Armstrong et al., 2007). Currently, the ADA recommends annual screening of DPN at diagnosis of T2D and 5 years after the diagnosis of type 1 diabetes (T1D) by neurological exam or monofilament testing, but there is no specific recommendation for pDPN (Pop-Busui et al., 2017).

There are currently no FDA approved therapies for DPN (Azmi et al., 2019a). Lifestyle interventions, including physical activity (Al-Kaabi et al., 2014) and avoidance of smoking (Al-

Mahroos and Al-Roomi, 2007, Tesfaye et al., 2005) are advised and optimization of glycemic control (Al-Mahroos and Al-Roomi, 2007, Boru et al., 2004), treatment of hypertension (Malik et al., 1998, Reja et al., 1995) and hyperlipidemia (Tesfaye et al., 2005, Smith and Singleton, 2013) may improve DPN. FDA approved medications for treating painful symptoms include duloxetine, pregabalin and tapentadol (Javed et al., 2015).

According to the International Diabetes Federation, the prevalence of diabetes in adults aged 20-79 years in Qatar was 15.5% in 2020 (IDF Middle East and North Africa Region, 2020,), which is almost two-fold greater than the 2019 reported prevalence of 8.3% in the rest of the world (International Diabetes Federation, 2019,). Given the high prevalence of diabetes, in 2015 Qatar launched the National Diabetes Strategy to improve the management of people with diabetes and its complications by establishing common clinical care pathways within and between primary and secondary health care. We have therefore applied the same methods and diagnostic criteria in patients with T2D to establish the prevalence and risk factors for DPN and pDPN in primary and secondary health care. We believe the findings of this study will be key to planning strategies to enable earlier diagnosis and optimal management of the often-forgotten complication of diabetic neuropathy, in Qatar and the region.

5.3 Materials and methods

This is a cross-sectional multi-center study. Subjects aged 18 - 85 years old with T2DM were enrolled from four primary health care (PHC) centers (Umm Ghuwailina, Al Khor, Al Daayen and Al Rayyan) and the only two National Diabetes centers in Qatar (Hamad General Hospital (HGH) and Al-Wakra Hospital). Subjects were randomly enrolled and screened for eligibility on the day they attended the clinic their diabetes review between June 2017 and February 2019. Exclusion criteria included T1D, other causes of neuropathy including severe vitamin B_{12} deficiency, chronic hypothyroidism and chemotherapy.

This study was approved by the Institutional Review Board (IRB) of Weill Cornell Medicine-Qatar (WCM-Q) and Hamad Medical Corporation (HMC). All subjects gave informed consent to take part in the study. The research adhered to the tenets of the declaration of Helsinki.

5.3.1 Demographic and metabolic measures

Gender, ethnicity, age, duration of diabetes and body mass index (BMI) were recorded. The average systolic (SBP) and diastolic blood pressure (DBP) of two readings were obtained from the subject's left arm while seated with the arm at heart level, using a standard zero mercury sphygmomanometer after 10-15 minutes of rest. A non-fasting blood sample was collected through venepuncture from each subject into EDTA tubes and transported within 2 hours to a central certified laboratory at HGH. Glycated haemoglobin (HbA1c), total cholesterol and triglyceride were measured by an autoanalyzer (Hitachi 747 autoanalyzer, Japan). Poor glycemic control was defined as HbA1c ≥9%. Hypertension was defined according to either an average SBP ≥140 mmHg and/or the use of anti-hypertensive medication, as described in the WHO/ISH Guidelines (Moser, 1999). Hyperlipidemia was defined according to a total cholesterol level ≥6.2 mmol/L and/or triglyceride level of ≥2.3 mmol/L or if the patient was treated with a statin. Obesity was classified according to WHO criteria with a BMI ≥30 kg/m^2 (Report of a WHO consultation, 2000). Current cigarette smoking was defined as having smoked at least one cigarette every day for ≥1 year preceding the study visit. Physical activity was defined as doing some physical activity including walking for ≥30 minutes/day, at least 3 times a week over the last year.

5.3.2 Assessment of diabetic neuropathy and painful neuropathy

The diagnosis of DPN was based on the presence of one or more neuropathic symptoms and impaired vibration perception threshold (VPT) in the feet. Subjective neurological symptoms such as burning pain, painful cold, electric shocks, tingling, pins and needles and numbness were acquired through a face-to-face interview with the investigators. VPT was measured by a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK) on the pulp of the large toe on both feet and the average value of three measurements was recorded in Volts (V) ranging from 0 - 50V. A VPT ≥15V was defined as impaired vibration perception consistent with the presence of DPN (Wiles et al., 1991) and a VPT ≥25V as high risk for diabetic foot ulceration (DFU) (Young et al., 1994).

Painful DPN was assessed using the Douleur Neuropathique en 4 (DN4) questionnaire in Arabic and English as previously described (Spallone et al., 2012). The DN4 questionnaire has been validated for its ability to distinguish neuropathic pain from non-neuropathic pain (Bouhassira et al., 2005) and in the Arabic version (Terkawi et al., 2017), and for pDPN

(Spallone et al., 2012). It consists of 10 questions: 7 questions relating to the pain description (burning, painful cold, electric shocks) and associated abnormal sensations (tingling, pins and needles, numbness, itching) and the other 3 neurological examination outcomes in the painful area for hypoesthesia to touch and pin prick using disposable examination pins and allodynia to brushing. The scoring is based on a yes (1 point) or no (0 point) answer and each question is equally weighted. A score ≥4 has a high sensitivity (80%) and specificity (92%) for pDPN (Spallone et al., 2012). The questionnaire was administered by the investigator in either English or Arabic. Previously diagnosed pDPN was self-reported. Medications for pDPN were recorded.

All investigators underwent a formal training session on the use and interpretation of the Neurothesiometer and DN4 questionnaire.

5.3.3 Statistical analysis

The recommended minimum sample size was 937 to estimate the prevalence of DPN and 1027 to estimate the prevalence of pDPN based on the assumption that the prevalence of DPN and pDPN was around 25% and 30% in a population of 5,000 patients with T2D in SHC with ±2.5 % the acceptance absolute deviation of sample rate from population rate and 95% confidence level.

The prevalence of DPN and pDPN across different demographics and risk factors as categorical variables were summarized using frequency distributions. Variables in patients with DPN or pDPN were compared between PHC and SHC using the Chi-squared test of independence.

Binary logistic regression analysis was performed with age, duration of diabetes, gender, poor glycemic control, obesity, hyperlipidemia, hypertension, physical activity, smoking, ethnicity and health care as independent variables, and DPN or pDPN as the dependent variable. Collinearity was tested to minimize its potential effect by selecting variables whose correlation coefficients was <0.7. The multiple logistic regression model included all variables with P value of ≤0.05 at the bivariate level. Adjusted odds ratios, their corresponding 95% confidence intervals (CI) and P value are presented.

All analyses were performed using IBM-SPSS (version 26; SPSS Inc, Armonk NY). A two-tailed P value of ≤0.05 was considered significant.

5.4 Results

5.4.1 Prevalence of DPN and pDPN in PHC compared to SHC (Figure 5.1 & Table 5.1)

1,386 subjects with T2D were recruited from primary (PHC) (n=297) and secondary health care (SHC) (n=1,089). The prevalence of DPN (14.8% vs 23.9%, P=0.001) was significantly lower in PHC compared to SHC. The percentage of patients undiagnosed with DPN was comparable (79.5% vs 82.3%, P=0.66) between PHC and SHC. The prevalence of pDPN (18.1% vs 37.5%, P<0.0001) and percentage of patients undiagnosed with pDPN (24.1% vs 71.5%, P<0.0001) was significantly lower in PHC compared to SHC. The mean VPT (10.4±7.2 V vs 12.5±9.4 V, P<0.0001), DN4 score (1.0±1.6 vs 2.5±2.6, P<0.0001) and percentage of patients with all neuropathic symptoms including burning, painful cold, electric shocks, tingling, pins and needles, numbness, itching were significantly lower in PHC compared to SHC (Table 5.2). Whilst no patients in PHC had a DFU, 6.2% had DFU in SHC. However, the prevalence of those at high risk for DFU was comparable (31.8% vs 40.0%, P=0.3) between PHC and SHC.

Figure 5.1. Prevalence of diabetic peripheral neuropathy (DPN), painful diabetic neuropathy (pDPN), undiagnosed DPN and pDPN in type 2 diabetes in primary and secondary health care.

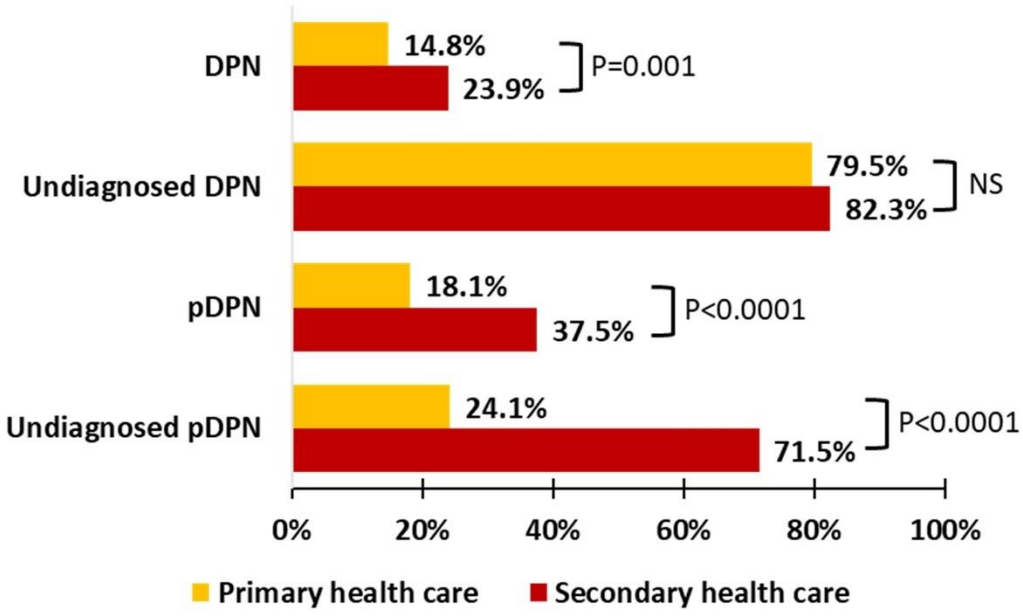

Table 5.1. Prevalence of diabetic peripheral neuropathy (DPN), painful diabetic neuropathy (pDPN), undiagnosed DPN and pDPN and those at high risk of DFU and their risk factors in type 2 diabetes in primary and secondary health care.

		Primary health care		Secondary health care		P value PHC vs SHC
Diabetic peripheral neuropathy		44/297	14.8%	260/1089	23.9%	0.001
High risk for diabetic foot ulceration		14/44	31.8%	104/260	40.0%	0.30
Diabetic foot ulcer		0/44	0.0%	16/260	6.2%	0.13
Painful diabetic neuropathy		54/298	18.1%	410/1092	37.5%	<0.0001
Undiagnosed cases						
Undiagnosed diabetic peripheral neuropathy		35/44	79.5%	214/260	82.3%	0.66
Undiagnosed painful diabetic neuropathy		13/54	24.1%	293/410	71.5%	<0.0001
Risk factors						
Age	20-50 years	88/295	29.8%a	445/1073	41.5%b	0.001
	51-60 years	117/295	39.7%a	379/1073	35.3%a	
	>60 years	90/295	30.5%a	249/1073	23.2%b	
Duration of diabetes	≤10 years	204/296	68.9%a	690/1080	63.9%a	0.26
	11-20 years	73/296	24.7%a	303/1080	28.1%a	
	>20 years	19/296	6.4%a	87/1080	8.1%a	
Lifestyle modifiable risk factors						
Physical activity		158/275	57.5%a	326/854	38.2%b	<0.0001
Smoking		27/274	9.9%	157/909	17.3%	0.003
Cardiovascular modifiable risk factors						
Poor glycemic control		98/266	36.8%a	436/991	44.0%b	0.04
Hyperlipidemia		208/259	80.3%a	738/1008	73.2%b	0.02
Hypertension		176/274	64.2%a	669/1040	64.3%a	0.98
Obesity		87/213	40.8%a	510/957	53.3%b	0.001
Systolic blood pressure (mmHg)		131.7±15.5		132.5±18.0		0.42
Diastolic blood pressure (mmHg)		78.8±8.0		78.2±10.2		0.32
BMI (Kg/m^2)		29.8±5.4		31.5±7.4		0.0003
HbA1c (mmol/mol)		63.1±19.7		65.5±21.9		0.08
HbA1c (%)		7.9±1.8		8.1±2.0		0.08
Cholesterol (mmol/L)		3.9±1.0		4.4±1.2		<0.0001
Triglyceride (mmol/L)		1.8±1.0		1.8±1.2		0.96

Variables were summarized using means and standard deviations for numeric variables and frequency distribution for categorical variables. Continues and categorical variables were compared using unpaired t-test and x^2, respectively. Symbols: a and b in each variable, rows with similar symbols are not statistically significant and different symbols are significantly different.

Table 5.2. The mean vibration perception threshold, DN4 score and percentage of neuropathic symptoms in type 2 diabetes in primary (PHC) and secondary (SHC) health care.

	PHC	SHC	P value PHC vs SHC
Vibration perception threshold (Volts)	10.4±7.2	12.5±9.4	<0.0001
DN4 score	1.0±1.6	2.5±2.6	<0.0001
Burning pain (%)	22.6	46.7	<0.0001
Painful cold (%)	7.7	26.5	<0.0001
Electric shocks (%)	5.1	22.3	<0.0001
Tingling (%)	20.2	32.0	<0.0001
Pins and needles (%)	16.2	35.1	<0.0001
Numbness (%)	12.5	42.0	<0.0001
Itching (%)	7.8	16.7	<0.0001

Variables were summarized using means and standard deviations for numeric variables and frequency distribution for categorical variables. Continues and categorical variables were compared using unpaired t-test and x^2, respectively.

5.4.2 Risk factor management in PHC compared to SHC

More patients with T2D aged above 60 years (30.5% vs 23.2%, P=0.001) and less patients aged between 20-50 years (29.8% vs 41.5%, P=0.001) were under the care of PHC compared to SHC. The BMI (29.8 Kg/m^2 vs 31.5 Kg/m^2, P=0.0003) and percentage of patients with obesity (40.8% vs 53.3%, P=0.001) was significantly lower in PHC compared to SHC. The HbA1c was comparable, but the percentage of patients with poor glycemic control (36.8% vs 44.0%, P=0.04) was lower in PHC compared to SHC. In PHC the total cholesterol (3.9 mmol/L vs 4.4 mmol/L, P<0.0001) was lower and triglycerides were comparable compared to SHC. However, hyperlipidaemia was present in a significantly lower percentage of patients in SHC compared to PHC (73.2% vs 80.3%, P=0.02). The systolic and diastolic blood pressure and percentage of patients with hypertension was comparable between PHC and SHC. More patients undertook physical activity (57.5% vs 38.2%, P<0.0001) and less patients smoked cigarettes (9.9% vs 17.3%, P=0.003) in PHC compared to SHC.

5.4.3 Association of risk factors for DPN in PHC and SHC (Table 5.3)

The odds of developing DPN increased by 2.4 (95% CI 1.6 – 3.5) times in patients aged 51-60 years (P<0.0001) and 2.9 (95% CI 1.9 – 4.5) times in those aged >60 years compared to patients aged 20-50 years (P<0.0001) (Cramer's V = 0.22). The odds increased 2.2 (95% CI 1.6 – 3.0) times with 11-20 years of diabetes (P<0.0001) to 3.9 (95% CI 2.4 – 6.4) times with >20 years of diabetes (P<0.0001) compared to ≤10 years of diabetes (Cramer's V = 0.30). The odds for DPN was 1.4 (95% CI 1.1 – 2.0) times greater in men (P=0.02) (Cramer's V = 0.02). The odds increased 1.5 (95% CI 1.1 – 2.0) times in those with poor glycemic control (P=0.02, Cramer's V = 0.12) and 1.6 (95% CI 1.2 – 2.3) times in patients treated with insulin and other anti-diabetic therapy compared to patients treated with metformin and other anti-diabetic therapy (P=0.006, Cramer's V = 0.22). The odds increased 1.8 (95% CI 1.2 – 2.8) times in those with hyperlipidemia (P=0.006, Cramer's V = 0.13) and 1.5 (95% CI 1.0 – 2.2) times in those with hypertension (P=0.05, Cramer's V = 0.17). The association with obesity and ethnicity for DPN was lost after controlling for risk factors. However, even after adjusting for all risk factors the odds of developing DPN in SHC remained 2.1 times higher than in PHC (P=0.001).

Table 5.3. Predictors for diabetic peripheral neuropathy in primary and secondary health care.

Diabetic peripheral neuropathy		AOR	95% CI	P value
Gender	Male	1		
	Female	0.7	0.5 – 0.9	0.02
Ethnic groups	Arabs	1		
	South Asians	0.8	0.5 – 1.1	0.19
Age	20-50 years	1		
	51-60 years	2.4	1.6 – 3.5	<0.0001
	>60 years	2.9	1.9 – 4.5	<0.0001
Duration of diabetes	≤10 years	1		
	11-20 years	2.2	1.6 – 3.0	<0.0001
	>20 years	3.9	2.4 – 6.4	<0.0001
Poor glycemic control		1.5	1.1 – 2.0	0.02
Hyperlipidemia		1.8	1.2 – 2.8	0.006
Hypertension		1.5	1.0 – 2.2	0.05
Obesity		1.3	0.9 – 1.8	0.20
Anti-diabetic therapy	Metformin/plus	1		
	Insulin/plus	1.6	1.2 – 2.3	0.006
Primary health care		1		
Secondary health care		2.1	1.4 – 3.2	0.001

The multiple logistic regression model included all variables with P value of ≤0.05 at the bivariate level. Adjusted odds ratios, their corresponding 95% confidence intervals (CI) and P value are presented.

5.4.4 Association of risk factors for pDPN in PHC and SHC (Table 5.4)

The odds of developing pDPN was 1.5 times greater in patients aged >50 years (P=0.02) compared to those aged 20-50 years (P<0.0001). The odds increased from 2.2 times with 11-20 years of diabetes (P<0.0001) to 4.4 times with >20 years of diabetes (P<0.0001) compared to ≤10 years of diabetes. The odds also increased by 1.7 times in subjects treated with insulin/plus other anti-diabetic therapy compared to those treated with metformin/plus other anti-diabetic therapy (P<0.0001). The odds increased 1.6 times with obesity (P=0.002) and 1.4 times in Arabs compared to South Asians (P=0.03). However, the odds decreased by 1.7 times with physical activity (P=0.01). The association of poor glycemic control, hyperlipidemia, hypertension and gender with pDPN was lost after controlling for these risk factors. However, even after adjusting for all risk factors the odds of developing pDPN in SHC was 2.4 times higher than in PHC (P<0.0001).

Table 5.4. Predictors for diabetic painful neuropathy in primary and secondary health care.

Painful neuropathy		AOR	95% CI	P value
Gender	Male	1		
	Female	1.2	0.9 – 1.6	0.32
Ethnic groups	Arabs	1		
	South Asians	0.7	0.5 – 1.0	0.03
Age	20-50 years	1		
	51-60 years	1.5	1.1 – 2.0	0.02
	>60 years	1.5	1.1 – 2.2	0.02
Duration of diabetes	≤10 years	1		
	11-20 years	2.2	1.6 – 3.0	<0.0001
	>20 years	4.4	2.7 – 7.1	<0.0001
Poor glycemic control		1.2	0.9 – 1.6	0.2
Hyperlipidemia		1.1	0.8 – 1.5	0.58
Hypertension		1.3	0.9 – 1.8	0.13
Obesity		1.6	1.2 – 2.2	0.002
Physical activity		0.6	0.4 – 0.9	0.01
Anti-diabetic therapy	Metformin/plus other therapy	1		
	Insulin/plus	1.7	1.3 – 2.4	<0.0001
Primary health care		1		
Secondary health care		2.4	1.6 – 3.5	<0.0001

The multiple logistic regression model included all variables with P value of ≤0.05 at the bivariate level. Adjusted odds ratios, their corresponding 95% confidence intervals (CI) and P value are presented.

5.5 Discussion

This study shows that the prevalence of DPN is 1.6 times lower and pDPN is 2 times lower in primary health care compared to secondary health care. Furthermore, the percentage of patients with undiagnosed DPN (~80%) and those at risk of DFU (32-40%) was extremely high and comparable between PHC and SHC, despite the institution of national diabetes care pathways. DPN was associated with poor glycemic control, hyperlipidemia and hypertension, whereas pDPN was associated with obesity and was lower in patients undertaking physical activity at least 3 days per week. The higher prevalence of DPN and pDPN in SHC remained significant even after controlling for risk factors. This may partly be attributed to referral bias with more patients with poorer control of risk factors and diabetic complication being referred to SHC.

The DN4 questionnaire was chosen to define pDPN for three reasons: 1) Its diagnostic ability to distinguish neuropathic pain from non-neuropathic pain including osteoarthritis, inflammation and mechanical low back pain (common differentials, especially in PHC) for which it has been validated with 86% sensitivity and 83% specificity (Bouhassira et al., 2005); 2) Its diagnostic ability specifically for pDPN with 80% sensitivity and 92% specificity (Spallone et al., 2012), and 3) validation using the Arabic version showing 88% sensitivity and 75% specificity (Terkawi et al., 2017). The higher prevalence of pDPN compared to DPN may be attributed to the criteria used to define these conditions. pDPN was defined according to DN4, whereas DPN was based on symptoms and an elevated VPT (>15V).

There are currently no FDA approved therapies for DPN (Azmi et al., 2019a). However, screening annually for symptoms and signs of DPN starting at diagnosis of T2D is advocated on the basis that early management of risk factors for DPN may reduce the rate of disease progression and treatment to relieve neuropathic symptoms may improve the patient's quality of life (Pop-Busui et al., 2017). We have recently assessed the prevalence of DPN and pDPN in SHC in Qatar (Ponirakis et al., 2020b, Ponirakis et al., 2019b). This is the first study to compare the prevalence of DPN and pDPN in PHC and SHC using the same criteria and in the same population. The prevalence of DPN in both PHC and SHC in Qatar is lower compared to the prevalence in SHC in other countries e.g. 37% in Bahrain (Al-Mahroos and Al-Roomi, 2007), 60% in Turkey (Boru et al., 2004), 49% in Iran (Kiani et al., 2013), 45% in the US (Mold

et al., 2004), 32% in the UK (Young et al., 1993), 31% in Italy (Salvotelli et al., 2015) and 62% in China (Lu et al., 2010). The prevalence of pDPN is also lower in both SHC and PHC in Qatar compared to studies from SHC with a reported prevalence of 65% in Saudi Arabia (Halawa et al., 2010), 61% in Egypt (Jambart et al., 2011), 58% in Jordan and 54% in Lebanon.

Despite the implementation of a referral system from PHC to SHC in May 2011in Qatar, which is based on clinical need rather than on a "first come first served" basis and has improved the quality and provision of the national diabetes performance, this study confirms an alarmingly high prevalence of undiagnosed DPN in PHC and SHC (Ponirakis et al., 2020b, Ponirakis et al., 2019b). It highlights the considerable need to educate both patients and physicians on DPN and pDPN (Malik et al., 2020). This may explain why up to 25% of patients with diabetes in SHC in Qatar have foot problems (Al-Thani et al., 2019). Indications for referral of patients with T2D from PHC to SHC, include poorly controlled T2D, recurrent or severe hypoglycemia, DPN, diabetic retinopathy or nephropathy. Currently, DPN is not assessed systematically even using the 10-g monofilament which in itself identifies only those with advanced neuropathy (Pop-Busui et al., 2017). Given that one in four patients with DFU are at risk of amputation (Apelqvist and Agardh, 1992), this study highlights the need for the National Diabetes Strategy to implement annual DPN screening in PHC and SHC. This should be done using evidence based screening tests to detect incipient small fiber damage to detect sudomotor dysfunction using Sudoscan (Selvarajah et al., 2015) or Neuropad (Ponirakis et al., 2014) or vibration perception using a Neurothesiometer (Bril and Perkins, 2002a) or cold or warm perception thresholds using NerveCheck (Ponirakis et al., 2016); as opposed to monofilament testing, which is convenient but only detects advanced large fiber neuropathy. A common reason for the under-diagnosis of pDPN is that patients with symptoms are often unaware that the pain is related to DPN and do not report them to their physician (Daousi et al., 2004, Eichholz et al., 2017). Although, several screening questionnaires, including the DN4 (Spallone et al., 2012), the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) pain scale (Bennett, 2001), the Neuropathic Pain Scale (NPS)(Jensen et al., 2006), the Neuropathic Pain Symptom Inventory (NPSI) (Bouhassira et al., 2004), and the Diabetic Peripheral Neuropathic Pain Impact measure (DPNPI) (Brod et al., 2015) have been developed to rapidly diagnose pDPN they remain under-utilized. Reassuringly, we show a much lower prevalence of patients with undiagnosed pDPN in PHC which may reflect a more systematic approach to identify

neuropathic symptoms as part of a general screen for complications as opposed to SHC where there is no formal screening unless the physician refers for further assessment.

The lack of a European Medicines Agency (EMA) and FDA approved therapy for DPN often creates a negative attitude on the need to diagnose early DPN (Malik et al., 2017). However, our study has identified a range of modifiable risk factors for DPN including poor glycemic control (Al-Mahroos and Al-Roomi, 2007, Boru et al., 2004), hypertension (Malik et al., 1998, Reja et al., 1995) and hyperlipidemia (Tesfaye et al., 2005, Smith and Singleton, 2013) and for pDPN e.g. obesity (Jambart et al., 2011, Van Acker et al., 2009, Ziegler et al., 2018, Aslam et al., 2015) and reduced physical activity (Ziegler et al., 2009, Smith et al., 2006). Although, intensive glycemic control is advocated, the data for an impact on DPN in T2D are limited (Ohkubo et al., 1995, Ismail-Beigi et al., 2010, Pop-Busui et al., 2013) and other cardiovascular risk factors such as hypertension, hyperlipidemia and obesity may play a more important role. Indeed, treatment with angiotensin converting enzyme (ACE) inhibitors (Malik et al., 1998, Reja et al., 1995), statins (Davis et al., 2008, Villegas-Rivera et al., 2015) or glucagon-like peptide 1 (GLP-1) receptor agonists (Kan et al., 2012, Himeno et al., 2011, Ponirakis et al., 2020a) may have a beneficial effect on DPN.

A limitation of this study is the relatively small number of participants from PHC, thus limiting the generalisability of the findings. A further limitation is the cross-sectional design of this study which limits the predictive validity of the observed associations between the various risk factors with DPN and pDPN. Another limitation is the reliability and validity of VPT which like all psychophysical tests relies on standardization and the participant's concentration and ability to detect a sensation. Whilst we show associations of DPN and pDPN with risk factors, the cross-sectional design of this study limits the predictive validity of these risk factors.

In conclusion, this study has identified a lower prevalence of DPN and pDPN in PHC compared to SHC, which may be attributed to better overall risk factor control in PHC and referral bias due to patients who are poorly managed with complications being referred to SHC. Alarmingly, an equally high proportion approximately 80% of patients with DPN were undiagnosed in both PHC and SHC, highlighting the need for the National Diabetes Strategy to implement annual DPN screening. The identification of hyperglycemia, hyperlipidemia and hypertension as modifiable risk factors for DPN and obesity and physical activity as modifiable

risk factors of pDPN provide a robust argument to establish protocols for the early diagnosis and management of DPN and pDPN.

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Chapter 6: Hypertension Contributes to Neuropathy in Patients With Type 1 Diabetes

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6.1 Abstract

Introduction: Diabetic peripheral neuropathy (DPN) can lead to foot ulceration and amputation. There are currently no disease modifying therapies for DPN. Previous studies of blood pressure lowering therapy have shown an improvement in some but not other measures of DPN.

Objectives: The aim of this study was to determine if hypertension contributes to DPN in patients with type 1 diabetes (T1D).

Methods: Subjects with T1D (n=70) and controls (n=78) aged 18-85 years were recruited from the Manchester Diabetes Centre, Manchester Royal Infirmary and the NIHR Wellcome Trust Clinical Research Facility. Subjects underwent a comprehensive assessment of DPN.

Results: Hypertension was present in 40/70 T1D subjects and 20/78 controls. Hypertension was associated with abnormal nerve conduction parameters (P=0.03-<0.001), increased vibration perception threshold (P=0.01) and reduced corneal nerve fiber density and length (P=0.02) in subjects with T1D. However, after adjusting for confounding factors only tibial compound motor action potential and nerve conduction velocity were associated with hypertension (P=0.03) and systolic blood pressure (P<0.01-<0.0001). Hypertension had no effect on neuropathy in subjects without diabetes.

Conclusions: This study shows that hypertension is associated with impaired nerve conduction in T1D. It supports previous small trials showing that ACE inhibitors improve nerve conduction and advocates the need for larger clinical trials with blood pressure lowering agents in DPN.

6.2 Introduction

There are currently no European Medicines Agency (EMA) and FDA approved treatments for diabetic peripheral neuropathy (DPN) (Pop-Busui et al., 2017). Whilst tight glycemic control is advocated for the treatment of DPN, it has only been shown to limit progression of neuropathy in patients with type 1 diabetes mellitus (T1D) and has shown no benefit in patients with type 2 diabetes mellitus (T2D) (Callaghan et al., 2012). However, clinical and experimental studies suggest that hypertension is an independent risk factor for DPN in patients with T1D (Tesfaye et al., 2005, Forrest et al., 1997, Cavusoglu et al., 2015, Elliott et al., 2009, Sanada et al., 2015, Gregory et al., 2012) and T2D (Cardoso et al., 2015, De Visser et al., 2014, Kesavamoorthy et al., 2015, Yang et al., 2015). In relation to the underlying pathophysiology, we have previously demonstrated loss of myogenic tone and vascular hypertrophy in resistance vessels of hypertensive patients with T2D (Schofield et al., 2002), with partial amelioration of these abnormalities after improved glycemic control (Greenstein et al., 2009) or treatment with the angiotensin-receptor blocker Candesartan (Malik et al., 2005).

Detailed preclinical studies suggest that hypertension predominantly affects the myelinated fibers. Hypertensive STZ rats with diabetes show myelinated fiber abnormalities (Sanada et al., 2015). Spontaneously hypertensive rats with diabetes show a reduction in sciatic nerve blood flow with a reduction in motor and sensory nerve conduction velocity and myelinated fiber density, but no loss of intraepidermal nerve fibers (Gregory et al., 2012). In a hypertensive T2D model, there was a reduction in sensory nerve conduction velocity and increased expression of matrix metalloproteinase at sites of myelin thinning (De Visser et al., 2014). In non-diabetic hypertensive rats impaired epineurial arteriolar function was shown to contribute to reduced endoneurial perfusion and neuropathy (Yorek, 2015) as well as axonal atrophy and myelin splitting with endoneurial microangiopathy (Nukada et al., 2016). However, treatment with Fosinopril prevented the development and maintenance of tactile allodynia (Araiza-Saldana et al., 2015) and a combination of Enalapril, α-lipoic acid and menhaden oil improved thermal hypoalgesia, intraepidermal nerve fiber profiles and corneal sub-basal nerve fiber length in a normotensive T2D model (Davidson et al., 2015). These improvements were related to improved vascular relaxation to acetylcholine and calcitonin

gene-related peptide in sciatic nerve epineurial arterioles. Recently, sacubitril/valsartan, a combination drug containing a neprilysin inhibitor and angiotensin II receptor blocker has been shown to prevent and reverse nerve conduction and intraepidermal and corneal nerve abnormalities in type 2 diabetic rats (Davidson et al., 2018).

We have shown that treatment of diabetic patients with the angiotensin converting enzyme (ACE) inhibitor Trandolapril, improved nerve conduction, but had no impact on neuropathic symptoms/deficits, vibration perception or autonomic function (Malik et al., 1998). Other studies have reported a significant improvement in nerve conduction, neuropathic symptoms and thermal thresholds in hypertensive patients with diabetes treated with an ACE inhibitor (Ruggenenti et al., 2011, Reja et al., 1995). Treatment of normotensive patients with DPN with the angiotensin-receptor blocker Losartan for 12 weeks did not show an improvement in NCS (Kubba et al., 2003). In the NATHAN-1 trial, patients treated with α-lipoic acid on ACE inhibitors showed improved heart rate variability (DB-HRV) (Ziegler et al., 2016).

We have undertaken a detailed study to identify the impact of hypertension on both large and small fiber measures of DPN in patients with T1D. We believe this may explain the disparate results of previous studies assessing the benefits of blood pressure lowering agents on DPN. It also helps to identify the neuropathy endpoints which should be used to determine the efficacy of blood pressure lowering therapies in DPN.

6.3 Methods

Participants with type 1 diabetes mellitus (T1D) and controls without diabetes aged 18-85 years were recruited from the Manchester Diabetes Centre, Manchester Royal Infirmary and the NIHR Wellcome Trust Clinical Research Facility. The study was performed at the NIHR Wellcome Trust Clinical Research Facility.

Exclusion criteria included corneal trauma/ dystrophy, corneal surgery in the last 6 months, vitamin B_{12} deficiency, hypothyroidism, neuropathy from non-diabetic causes and diabetes or impaired glucose tolerance in the control group. This study was approved by the Local Research Ethics committee and all participants gave informed consent to take part in the study. The research adhered to the tenets of the declaration of Helsinki.

6.3.1 Blood pressure measurement

Blood pressure (BP) was assessed in all participants on the non-dominant arm, assuring correct cuff size, with an automated device DINAMAP PRO 400 (Critikon, Florida, US) in the sitting position after 5 minutes rest on two occasions. Hypertension was defined according to either an average systolic blood pressure (SBP) ≥ 140 mmHg from two sets of measurement as described in the WHO/ISH Guidelines or if subjects were on anti-hypertensive treatment.

6.3.2 Clinical measures

All participants underwent assessment of body mass index (BMI), glycated haemoglobin (HbA1c), cholesterol and triglycerides.

6.3.3 Neuropathy and neuropathic pain assessment

DPN was diagnosed according to the criteria established by the Toronto Diabetic Neuropathy Expert Group (Tesfaye et al., 2010) These criteria include neuropathy symptoms or neuropathy signs and an abnormality of nerve conduction studies (NCS) or a validated measure of small fiber neuropathy (corneal nerve fiber length) (Petropoulos et al., 2013a, Chen et al., 2015). The assessments were performed by different researchers who were blinded to subject group and the researchers were acting independently, with no exchange of results during the study.

Neuropathic symptoms were assessed using the DNS score (Meijer et al., 2002), a four-item validated symptom score for symptoms of unsteadiness in walking, neuropathic pain, paraesthesia, and numbness, giving a maximum score of 4 points, with a score of ≥ 1 defining the presence of neuropathic symptoms. Neuropathy signs were defined using the NDS (Young et al., 1993) that includes examination of vibration perception using a 128-Hz tuning fork, pin-prick on the tip of the large toe, temperature perceptions in the dorsum of the feet, and the presence or absence of ankle reflexes. Subjects scoring > 2/10 were considered to have signs of neuropathy.

Neuropathic pain was defined by a combination of deficits with an NDS score >2 and the presence of painful symptoms using the McGill Pain Questionnaire to assess the type of pain using descriptors such as throbbing, shooting, distressing, excruciating etc. (Melzack, 1975).

6.3.4 Corneal Confocal Microscopy

Participants underwent examination with the Heidelberg Retina Tomograph (HRT III RCM) *in vivo* corneal confocal microscope (Heidelberg Engineering GmbH, Heidelberg, Germany) using our established methodology (Petropoulos et al., 2013c). Three CCM images from the sub-basal nerve plexus in the central cornea were captured per eye. Corneal nerve fiber density (CNFD), number of main nerve fibers per mm^2 (no./mm^2), branch density (CNBD), number of nerve branches per mm^2 (no./mm^2), and fiber length (CNFL), length of nerve fibers per mm^2 (mm/mm^2) were quantified manually using CCMetrics, a validated image analysis software (Petropoulos et al., 2013c). The cut-off values of CNFD (≥19 no./mm^2), CNBD (≥42 no./mm^2) and CNFL (≥16 mm/mm^2) were based on the study by Petropoulos et al. 2014 that assessed the validity of CCM in diagnosing DPN (Petropoulos et al., 2014).

6.3.5 Intraepidermal Nerve Fiber Density

A 3 mm punch skin biopsy was taken from the dorsum of the foot under 1% lidocaine local anaesthesia. Skin samples were immediately fixed in 4% (wt/vol.) paraformaldehyde for 24 hours and then cryoprotected in sucrose, frozen and cut into 50 μm sections. Immunohistochemistry was performed as previously described (Azmi et al., 2015). A Zeiss AxioImager M2 microscope (Carl Zeiss, Jena, Germany) was used to quantify intra epidermal nerve fiber density (IENFD), which is the total number of nerve fibers per millimetre length of epidermis (no./mm), in accordance with established criteria (Lauria et al., 2010b).

6.3.6 Autonomic neuropathy

Cardiovascular autonomic neuropathy (CAN) was evaluated using the ANX 3.0 autonomic nervous system monitoring device (ANSAR Medical Technologies Inc. Philadelphia, US) (Orlov et al., 2012). Deep Breathing-Heart Rate Variability (DB-HRV) was assessed by R-R interval variation via surface electrodes over 1 minute at a frequency of 6 breaths/minute.

Peripheral autonomic dysfunction was assessed using the Neuropad (Miro Verbandstoffe, Wiehl-Drabenderhöhe, Germany) applied to the plantar aspect of the 1st metatarsal head for 10 minutes, followed by quantification of the percentage colour change of the Neuropad.

6.3.7 Quantitative sensory testing

Quantitative sensory testing (QST) included measurement of vibration perception threshold (VPT) on the tip of the large toe using the Neurothesiometer (Horwell, Scientific Laboratory Supplies, Nottingham, UK) and warm and cold perception thresholds (WPT & CPT) on the dorsum of the left foot using the method of limits with the MEDOC *TSA II* (Medoc Ltd. Ramat Yishai 30095, Israel).

6.3.8 Nerve conduction

Electrodiagnostic studies were undertaken using a Dantec "Keypoint" system (Dantec Dynamics Ltd. Bristol, UK) equipped with a DISA temperature regulator to keep lower limb temperature constantly between 32 and 35^{o}C. Sural sensory nerve action potential (SNAP), sural nerve conduction velocity (SNCV), tibial compound motor action potential (TCMAP), tibial motor nerve conduction velocity (TMNCV), peroneal compound motor action potential (PCMAP) and peroneal motor nerve conduction velocity (PMNCV) were assessed in the right lower limb by a consultant neurophysiologist. Sural sensory responses were measured using a bipolar bar electrode (inter-electrode distance 3 cm) attached over the sural nerve at the lateral malleolus. Stimulation was performed 140 mm proximal to the active recording electrode in the calf. Abnormal nerve conduction was defined based on two abnormal nerve conduction velocities of either SNCV, TMNCV or PMNCV. The cut-off values of the nerve conduction velocities were defined on the - 2SD from the mean based on our control population.

6.3.9 Statistical analysis

The sample size needed to detect significant differences in CCM and NCS between the groups was calculated from our previously published data (Chen et al., 2015). Given a reported difference in population means of 8 no./mm^2 for CNFD and 5 m/s for PMNCV, estimated standard deviation for within group differences of 7 for CNFD and 3 for PMNCV, and aiming for a study power of 80% and an alpha of 0.05, we estimated that ~17 participants for each group would be needed to conduct this study.

Differences between normotensive and hypertensive groups in continuous variables were compared using unpaired t-test or categorical variables when the distribution had skewness of < -1 or > 1 with Mann-Whitney test. Categorical variables were compared using chi-square

or Fisher's exact test (when sizes were less than 5). Data are expressed, based on the scale of measurements, as mean [standard deviation (SD)] or frequency distribution. This analysis was done separately for the control group and the diabetic group. The analysis was performed using StatsDirect version 3.0.

The above analysis was repeated while adjusting for baseline imbalances between the two groups (normotensive and hypertensive) using multiple linear regression analysis for continuous variables and multiple logistic regression analysis for categorical variables. Assumptions of linear regression were satisfied for normality, collinearity and outliers. Additionally, residual plots were used to determine for linearity, normality, constant variance and independence. Finally, a multiple linear regression model was created to test the association between SPB and neuropathy measures adjusting for potential confounders. The analysis was performed using SPSS (version 23; SPSS Inc, Chicago).

A two-tailed P value of ≤0.05 was considered significant.

6.4 Results

6.4.1 Clinical data (Table 6.1)

The demographic and clinical characteristics are summarized in Table 6.1. 58 normotensive controls, 20 hypertensive controls, 30 normotensive and 40 hypertensive T1D participants were studied. All four groups had comparable age and gender. The duration of diabetes was comparable between hypertensive and normotensive T1D participants. Both systolic (SBP) and diastolic blood pressure (DBP) were significantly higher in the hypertensive controls (151.35±12.17 mmHg vs 121.58±12.63 mmHg, P<0.0001 and 82.15±9.75 vs 70.54±8.19 mmHg, P<0.0001) and subjects with T1D (142.58±17.74 mmHg vs 117.89±10.19 mmHg, P<0.0001 and 74.08±9.83 vs 67.68±8.10 mmHg, P<0.01) compared to normotensive controls and subjects with T1D, respectively. Hypertensive controls had significantly higher cholesterol levels compared to normotensive controls (5.54 ±0.75 mmol/l vs 4.98 ±0.79 mmol/l, *P*=0.01), but HbA1c, triglycerides and BMI were comparable. Hypertensive T1D participants had significantly higher triglycerides (1.39 ±0.73 mmol/l vs 0.95 ±0.53 mmol/l, *P*<0.01) and BMI (27.71 ±3.70 Kg/m^2 vs 25.55 ±4.12 Kg/m^2, *P*<0.05) compared to normotensive T1D participants, but HbA1c and cholesterol were comparable.

Table 6.1. Demographic characteristics of the study population.

	Control			T1D		
	Normotensive	Hypertensive	P value	Normotensive	Hypertensive	P value
n	58	20		30	40	
Age, years	47.84±11.91	53.35±13.40	NS	44.19±11.11	49.52±12.19	NS
Gender (F, M), n	29 29	10 10	NS	16 14	13 27	NS
SBP, mmHg	121.58±12.63	151.35±12.17	<0.0001	117.89±10.19	142.58±17.74	<0.0001
DBP, mmHg	70.54±8.19	82.15±9.75	<0.0001	67.68±8.10	74.08±9.83	<0.01
Diabetes duration, years	N/A	N/A		27.23 ±12.89	31.63±15.95	NS
HbA1c, %	5.63±0.34	5.58±0.33	NS	7.89±1.86	8.30±1.40	NS
HbA1c, mmol/l	38.06±3.72	37.31±3.57		66.53±14.86	67.24±15.35	
Chol. mmol/l	4.98±0.79	5.54±0.75	0.01	4.40±0.88	4.24±0.90	NS
Trig. mmol/l	1.42±0.74	1.70±0.73	NS	0.95±0.53	1.39±0.73	<0.01
BMI, Kg/m^2	26.72±4.84	29.01±4.46	NS	25.55±4.12	27.71±3.70	<0.05

Comparing the characteristics between normotensive vs hypertensive control subjects, and normotensive vs hypertensive T1D subjects. Values presented as mean ±standard deviation unless otherwise stated. Unpaired t-test was applied to assess for parametric data. Abbreviations: SBP=systolic blood pressure, DBP=diastolic blood pressure.

6.4.2 Neuropathy and neuropathic pain (Table 6.2)

The neuropathy findings between normotensive and hypertensive subjects in the T1D and control group are summarized in Table 6.2. The prevalence of DPN (53.8% vs 51.7%) and painful DPN (38.5% vs 23.3%) were comparable between patients with T1D with and without hypertension, respectively. There were no difference in the prevalence of DPN (10.0% vs 7.0%) and painful DPN (5.3% vs 1.8%) between the hypertensive and normotensive controls.

Table 6.2. Neuropathy measures in the study population.

	Control		P value/ P value*	T1D		P value/ P value*
	Normotensive	Hypertensive		Normotensive	Hypertensive	
n	58	20		30	40	
Neuropathy, n (%)	4 (7.0)	2 (10.0)	NS/NS	15 (51.7)	21 (53.8)	NS/NS
Neuropathic pain, n (%)	1 (1.8)	1 (5.3)	NS/NS	7 (23.3)	15 (38.5)	NS/NS
Nerve fiber morphology						
CNFD, no./mm^2	36.99±6.39	35.42±6.69	NS/NS	27.61±7.60	22.04±10.33	0.02/NS
CNBD, no./mm^2	90.95±40.35	84.07±28.65	NS/NS	60.80±30.55	46.83±31.86	NS/NS
CNFL, mm/mm^2	25.99±5.50	25.26±5.10	NS/NS	20.28±5.58	16.40±6.83	0.02/NS
IENFD, no./mm	9.49±4.21	10.17±1.76	NS/NS	6.89±4.43	5.12±3.77	NS/NS
Autonomic neuropathy						
HRV-DB, beats/minute	28.88±12.60	27.89±10.97	NS/NS	25.49±10.68	20.11±10.58	NS/NS
Neuropad, %	84.33±23.16	89.25±14.38	NS/NS	76.46±28.71	70.92±34.31	NS/NS
Quantitative sensory testings						
VPT, V	6.24±5.11	7.27±5.40	NS/NS	9.40±7.04	15.37±11.38	0.01*/NS
CPT, ^{o}C	28.43±2.06	27.49±2.13	NS/NS	24.51±6.66	25.37±4.50	NS/0.02
WPT, ^{o}C	37.34±3.32	36.63±2.13	NS/NS	39.62±4.06	40.59±4.37	NS/NS
Nerve conduction						
SNAP, µV	20.82±10.43	14.87±6.92	0.01/NS	11.33±7.31	6.95±6.75	0.01/NS
SNCV, m/s	51.08±4.81	49.49±4.07	NS/NS	41.98±10.31	39.63±7.84	NS/NS
TCMAP, mV	12.69±4.18	10.92±4.19	NS/NS	10.87±4.10	6.38±4.62	<0.001/0.03
TMNCV, m/s	48.96±3.20	48.57±3.95	NS/NS	44.92±4.08	39.39±5.82	<0.001/0.03
PCMAP, mV	5.12±2.04	4.66±2.22	NS/NS	3.76±2.20	2.56±2.06	0.03/NS
PMNCV, m/s	49.03±3.63	47.00±4.02	NS/NS	41.87±6.93	39.06±6.52	NS/NS

Characteristics of normotensive vs hypertensive control subjects, and normotensive vs hypertensive T1D subjects. Values presented as mean ±standard deviation unless otherwise stated. Unpaired t-test was applied to assess parametric data. (*) Mann-Whitney test was applied to assess non-parametric data. P value* were adjusted for baseline imbalances in each group according to table 6.1. Abbreviations: CNFD=corneal nerve fiber density, CNBD=corneal nerve branch density and CNFL=corneal nerve fiber length, IENFD=intra-epidermal nerve fiber density, HRV-DB =heart rate variability with deep breathing, VPT=vibration perception threshold, CPT=cold perception threshold, WPT=warm perception threshold, SNAP=sural sensory nerve action potential, SNCV=sural nerve conduction velocity, TCMAP=tibial compound motor action potential, TMNCV=tibial motor nerve conduction velocity, PCMAP=peroneal compound motor action potential and PMNCV=peroneal motor nerve conduction velocity.

6.4.3 Corneal and intra epidermal nerve fiber morphology

The T1D group with hypertension had a significantly lower CNFD (22.04 [SD 10.33] no./mm^2 vs 27.61 ± 7.60 no./mm^2, *P*=0.02) and CNFL (16.40 ± 6.83 mm/mm^2 vs 20.28 ± 5.58 mm/mm^2, *P*=0.02) compared to the normotensive group. However, these significant differences were lost after adjusting for age, gender, triglycerides and BMI. There was no difference in CNBD (46.83 ± 31.86 vs 60.80 ± 30.55 no./mm^2) and intra epidermal nerve fiber density (IENFD) (5.12 ± 3.77 vs 6.89 ± 4.43 no./mm^2) between the normotensive and hypertensive T1D groups (Table 6.2, Figure 6.1 and 6.2). CNFD, CNBD, CNFL and IENFD were comparable between the normotensive and hypertensive control groups.

Figure 6.1. Corneal confocal microscopy (CCM) images of the sub-basal nerve plexus in a normotensive control (A), hypertensive control (B) showing normal corneal nerve morphology and a normotensive T1D patient (C) and hypertensive T1D patient (D) showing a reduction in corneal nerve fiber density, branch density and length.

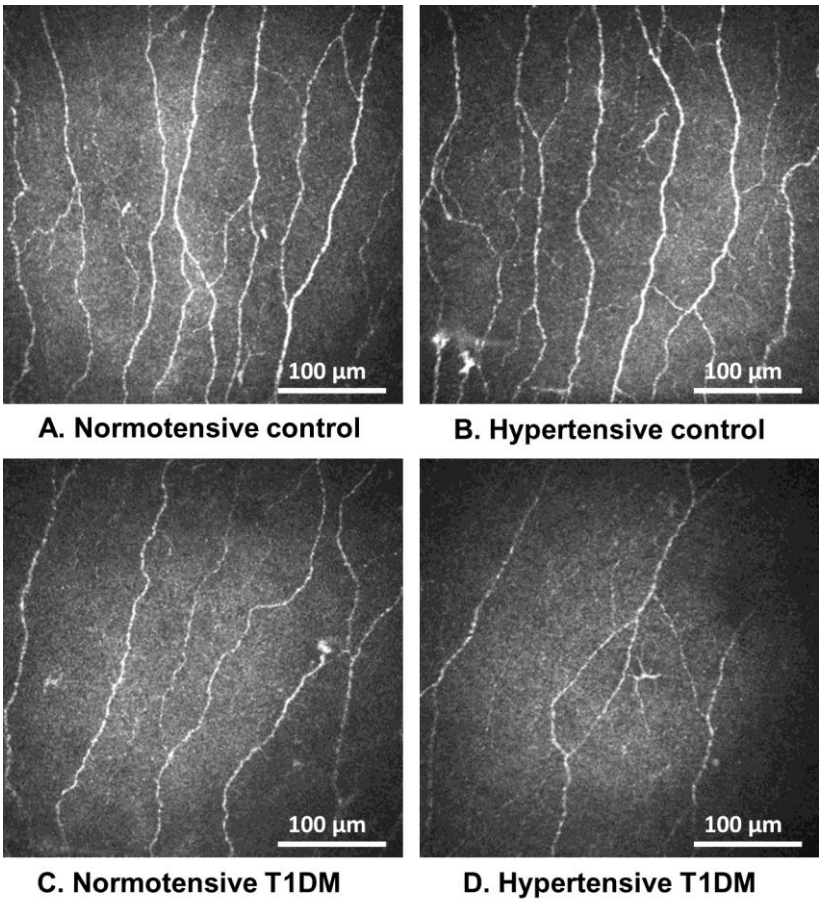
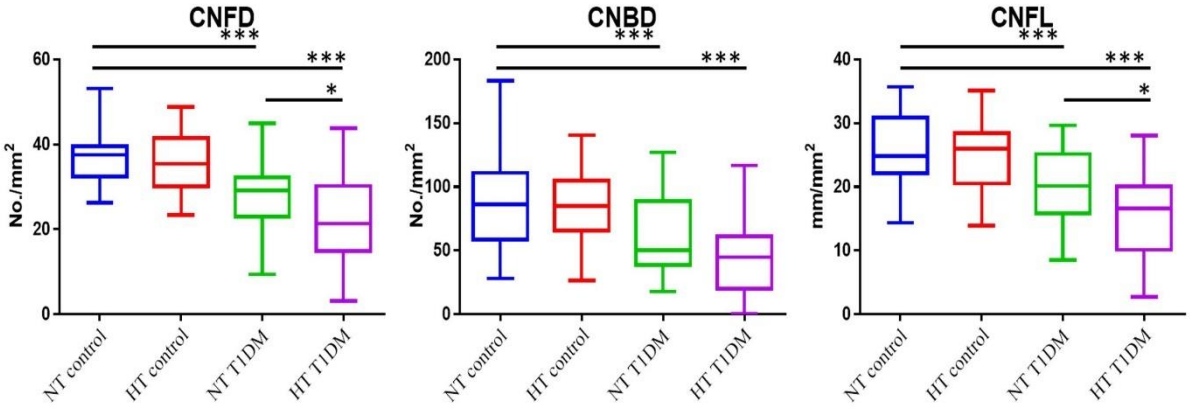


Figure 6.2. Corneal nerve morphology in normotensive controls (blue), hypertensive controls (red), normotensive T1D participants (green) and hypertensive T1D participants (purple). Box plots of corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD) and corneal nerve fiber length (CNFL). The line in the middle of the boxes represents the median and the boxes extend from the 25th to 75th percentiles. The whiskers extend from the highest to the lowest value. Significant differences between the groups were expressed as *P≤0.01 and *** P< 0.0001.



6.4.4 Autonomic neuropathy

There were no differences in deep breathing heart rate variability (DB-HRV) and Neuropad response between the T1D and control participants with and without hypertension.

6.4.5 Quantitative sensory testing (QST)

Vibration perception threshold (VPT) was significantly higher in hypertensive (15.37 ± 11.38 compared to normotensive (9.40 ± 7.04 V, P=0.01) patients with T1D, but the difference was no longer significant after adjusting for age, gender, triglycerides and BMI. The cold (CPT) and warm perception threshold (WPT) were comparable. However, after adjusting for baseline imbalances the CPT was significantly higher in the hypertensive group (P=0.02). There were no differences in VPT, CPT or WPT between the normotensive and hypertensive control groups.

6.4.6 Nerve conduction studies

T1D patients with hypertension had a significantly lower sural sensory nerve action potential (SNAP) (6.95 ± 6.75 μV vs 11.33 ± 7.31 μV, *P*=0.01), tibial compound motor action potential (TCMAP) (6.38 ± 4.62 mV vs 10.87 ± 4.10 mV, *P*<0.001), tibial motor nerve conduction velocity (TMNCV) (39.39 ± 5.82 m/s vs 44.92 ± 4.08 m/s, *P*<0.001) and peroneal compound motor action potential (PCMAP) (2.56 ± 2.06 mV vs 3.76 ± 2.20 mV, *P*=0.03). However, after adjusting for age, gender, triglycerides and BMI the differences were no longer significant apart from TCMAP and TMNCV. Sural nerve conduction velocity (SNCV) (39.63 ± 7.84 m/s vs 41.98 ± 10.31 m/s) and peroneal motor nerve conduction velocity (PMNCV) (39.06 ± 6.52 m/s vs 41.87 ± 6.93 m/s) were comparable between the two subgroups. In the control group, only SNAP (14.87 ± 6.92 μV vs 21.82 ± 10.43 μV, *P*=0.01) was lower in the hypertensive compared to the normotensive group but the difference was no longer significant after adjusting for age, gender and cholesterol and SNCV, TCMAP, TMNCV, PCMAP and PMNCV were comparable.

6.4.7 Association between neuropathy and systolic blood pressure (Table 6.3)

Simple linear regression analysis shows that all measures of DPN including CNFD, CNFL, HRV, SNAP, SNCV, TCMAP, TMNCV, PCMAP, PMNCV and VPT were associated with SBP in patients with T1D. However, after adjusting for confounding factors including age, gender, duration of diabetes, HbA1c, cholesterol, triglyceride and BMI, multiple linear regression analysis showed that only TCMAP (β=-1.12, *P*<0.0001, Cohen's d = 1.09) and TMNCV (β=-0.10, *P*<0.01, Cohen's d = 1.10) were independently associated with SBP (Table 6.3).

In the control group, simple linear regression analysis showed that all nerve conduction parameters apart from PCMAP were associated with systolic BP. However, after adjusting for confounding factors, only SNAP (β=-0.16, *P*=0.01) was independently associated with SBP.

Table 6.3. Multiple linear regression analysis showing the association between measures of neuropathy and systolic blood pressure in subjects with T1D after adjusting for confounding factors.

	Coefficient	95% Confidence Interval	P value
Corneal nerve morphology			
CNFD	-0.09	-0.20 to 0.02	NS
CNFL	-0.08	-0.16 to 0.003	NS
Cardiac autonomic neuropathy			
HRV	-0.02	-0.15 to 0.11	NS
Quantitative sensory testing			
VPT	0.08	-0.03 to 0.19	NS
Nerve conduction (NC)			
SNAP	-0.05	-0.13 to 0.03	NS
SNCV	-0.1	-0.21 to 0.02	NS
TCMAP	-0.12	-1.17 to -0.07	<0.0001
TMNCV	-0.10	-0.16 to -0.03	<0.01
PCMAP	-0.01	-0.04 to 0.01	NS
PMNCV	0.003	-0.08 to 0.08	NS

Variables affecting diabetic neuropathy were considered in the fitted model with a *P* value ≤0.05.

6.5 Discussion

This study shows that DPN is associated with hypertension and raised systolic blood pressure in T1D. It also shows that the association of hypertension with DPN measures varied between small and large fibers. This might explain as to why previous studies of blood pressure lowering therapy have shown an improvement in some but not other measures of diabetic neuropathy, although other factors including severity of DPN, diabetes duration, different measures of DPN or type of study could have contributed to conflicting results. We show that hypertension worsens deficits in NCS and vibration perception in subjects with T1D, indicating an abnormality of large nerve fibers, but is also associated with loss of corneal nerve fibers using CCM. This is clinically relevant as small nerve fibers are the earliest to be damaged and underlie the pathogenesis of foot ulceration (Breiner et al., 2014, Quattrini et al., 2007b, Quattrini et al., 2007a) and painful diabetic peripheral neuropathy (DPN) (Haanpaa et al., 2011). However, after adjusting for baseline imbalances including age, gender, triglyceride and BMI, only tibial compound motor action potential and motor nerve conduction velocity were affected by hypertension. Similarly, after adjusting for confounding factors including

age, gender, duration of diabetes, HbA1c, cholesterol, triglyceride and BMI, multiple linear regression analysis showed that only TCMAP and TMNCV remained independently associated with systolic blood pressure.

Given that there are no disease modifying therapies for DPN, this encourages the need for clinical trials of blood pressure lowering agents in DPN and provides direction for the endpoints which should be utilised in these trials. Both clinical and experimental studies have shown that treatment with an ACE inhibitor leads to an improvement in NCS (Malik et al., 1998, Ruggenenti et al., 2011, Reja et al., 1995, Davidson et al., 2015), but has no impact on symptoms, deficits, VPT or autonomic function. Indeed, we show that hypertension does not influence neuropathic symptoms or thermal thresholds, and therefore may not change. Istenes *et al.* (Istenes et al., 2008) reported an association between hypertension and cardiac autonomic neuropathy (CAN) in T2D, which is associated with silent myocardial ischemia, cardiac arrhythmias and cardio-respiratory instability (Vinik et al., 2003, Ziegler, 1994). In a study of T1D and T2D patients with CAN, 12 months of treatment with Quinapril, Losartan or a combination of both showed an improvement in CAN (Didangelos et al., 2006). However, in the present study we show a limited association between deep breathing heart rate variability (DB-HRV) and systolic blood pressure, which was lost after adjusting for age, gender, duration of diabetes, triglycerides and BMI. Additionally, there was no effect of hypertension on sudomotor dysfunction.

Limitations of this study include the use of a single as opposed to cumulative burden of blood pressure and glucose control on DPN and the relatively small numbers of subjects studied. We acknowledge that a cross-sectional study showing an association between hypertension and nerve conduction cannot imply cause and effect. However, a major strength of this study is the homogeneity of age, gender and duration of diabetes as well as the detailed neuropathy assessments, which have enabled us to identify the exact associations between hypertension and specific measures of neuropathy. It provides an explanation as to why some studies assessing the effect of blood pressure treatment have been positive, whist others have been negative, depending on the measures chosen to assess DPN.

This study shows that hypertension is associated with nerve conduction abnormalities in T1D but has no impact in subjects without diabetes. It also shows that the detrimental impact of

T1D on DPN may be mediated by hypertension on the myelinated fibers and by a number of metabolic risk factors including hyperglycemia, high triglycerides and obesity affecting the small fibers. These data suggest that nerve conduction studies should be used as the primary endpoints in clinical trials assessing the benefits of blood pressure lowering therapy on diabetic neuropathy.

6.6 Acknowledgements

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6.7 Funding

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Chapter 7: Metformin use is not associated with B_{12} deficiency or neuropathy in patients with Type 2 Diabetes in Qatar

Authors: Elhadd T, Ponirakis G, Dabbous Z, Siddique M, Chinnaiyan S, Malik R.A. **Frontiers of Endocrinology**. 2018 May 25;9:248. **DOI**: 10.3389/fendo.2018.00248

7.1 Abstract

Introduction: Metformin may lead to B_{12} deficiency and neuropathy. There are no published data on the prevalence of metformin related B_{12} deficiency and neuropathy in the Arabian Gulf.

Objectives: Determine whether metformin intake is associated with B_{12} deficiency and whether B_{12} deficiency is associated with diabetic peripheral neuropathy (DPN) and painful diabetic neuropathy.

Methods: Patients with Type 2 Diabetes (T2D) (n=362) attending outpatient clinics at HMC underwent assessment of B_{12} levels, the DN4 questionnaire, and vibration perception threshold (VPT).

Results: Comparing metformin to non-metformin users there were no differences in B_{12} levels, VPT or DN4. The prevalence of B_{12} deficiency (B_{12} < 133 pmol/l) was lower (P<0.01) in metformin (8%) compared to non-metformin (19%) users. Patients with B_{12} deficiency had a comparable prevalence and severity of sensory neuropathy and painful neuropathy to patients without B_{12} deficiency.

Conclusion: Serum B_{12} levels were comparable between metformin and non-metformin users with T2D in Qatar. T2D patients on metformin had a lower prevalence of B_{12} deficiency. Furthermore, the prevalence and severity of neuropathy and painful diabetic neuropathy were comparable between patients with and without B_{12} deficiency.

7.2 Introduction

Metformin remains first-line therapy in Type 2 Diabetes (T2D), with around 120 million users worldwide. It is increasingly used in overweight T2D patients and those with polycystic ovary syndrome (Viollet et al., 2012). Most international guidelines recommend metformin after lifestyle measures for T2D patients.

Metformin therapy was shown to be associated with a significant reduction in the level of vitamin B_{12} over 50 years ago (Berchtold et al., 1969, Tomkin et al., 1971). A number of observational and placebo-controlled studies have confirmed that metformin may reduce vitamin B_{12} levels (DeFronzo and Goodman, 1995, de Jager et al., 2010, de Groot-Kamphuis et al., 2013, Aroda et al., 2016, Kang et al., 2014, Reinstatler et al., 2012, Damiao et al., 2016). Indeed a recent study from Pakistan found that 29.7% of patients on metformin had B_{12} deficiency (Khan et al., 2017) and another study from Brazil showed that B_{12} deficiency occurred in 22.4% of patients with T2D on metformin, and was further reduced in those on PPI/H2-antagonists (Damiao et al., 2016). However, a recent meta-analysis showed that 10/17 studies found that metformin use led to B_{12} deficiency and in four prospective studies B_{12} was reduced by approximately 57pmol/L, within 6 weeks to 3 months of commencing metformin (Chapman et al., 2016).

A potential consequence of B_{12} deficiency is that it could directly result in neuropathy or exacerbate diabetic neuropathy. Indeed, the recent 2017 ADA position statement on diabetic neuropathy has emphasized the importance of excluding B_{12} deficiency in patients with diabetic neuropathy (Pop-Busui et al., 2017). However, there are conflicting reports on the association between metformin induced B_{12} deficiency and neuropathy, with some reports showing an association (Singh et al., 2013, Roy et al., 2016) whilst others have refuted this (Khan et al., 2017, Russo et al., 2016, Ahmed et al., 2016, Ma et al., 2015). Furthermore, in a recent study from Turkey, whilst the prevalence of B_{12} deficiency was 38.4% there was no difference in B_{12} levels in those with and without neuropathy (Olt and Oznas, 2017). Despite this there is wide spread administration of vitamin B_{12} therapy in patients in the Middle East and Far East, with a recent analysis from 5 teaching hospitals in Jordan, indicating that cyanocobalamin (B_{12}), was the 2nd most common injectable therapy after insulin (Al-Azayzih

et al., 2017). There are no published data on metformin related B_{12} deficiency or the relationship between B_{12} deficiency and diabetic neuropathy in the MENA region.

We have compared vitamin B_{12} levels in outpatients with T2D in Qatar, in relation to metformin use and further assessed for the prevalence and severity of painful neuropathy and sensory neuropathy in patients with B_{12} deficiency.

7.3 Materials and Methods

Participants with T2D (n=362) were recruited from the National Diabetes & Endocrine Centers in Al-Wakra Hospital and Hamad General Hospital. The study was performed between 6th March 2017 and 28th September 2017.

Exclusion criteria included patients with a prior history of pernicious anemia, chronic kidney disease, previous bariatric surgery, gastrectomy or small bowel resection for inflammatory bowel disease. This study was approved by the Institutional Review Board (IRB) of WCM-Q and HMC and all participants gave informed consent to take part in the study. The research adhered to the tenets of the declaration of Helsinki.

7.3.1 Demographic and blood measures

Data including age, duration of diabetes, blood pressure, body mass index (BMI) and medications including metformin were recorded. HbA1c, lipid profile, renal function and serum B_{12} were assessed.

7.3.2 B_{12} assay

Blood was drawn directly into a dedicated evacuated tube (BD Diagnostic – Preanalytical Systems, Oxford, UK) and centrifuged at 3500g for 10 minutes and serum analysed immediately or stored at -20 degrees centigrade until analysis on Beckman Dxi 600 (Beckman Coulter Inc, Brea, CA USA). The Vitamin B_{12} assay is a competitive-binding immunoenzymatic assay. The amount of analyte in the sample was determined by means of a stored, multipoint calibration curve (Beckman Coulter Assay Manual 2015, Beckman Coulter Inc, Brea, CA). Analytical Sensitivity <50 pg/ml, traceability; traceable to an internal standard manufactured

using the purified cyanocobalamin. Assay precision: 4.8 – 11.4%. B_{12} levels <133 pmol/l were considered deficient.

7.3.3 Diabetic peripheral neuropathy assessment

Vibration perception threshold (VPT) was measured on the pulp of the large toe with a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK). The test was repeated three times and the average value was recorded. VPT at a cut-off point ≥ 15 V was defined as diabetic peripheral neuropathy (DPN) (Wiles et al., 1991).

7.3.4 Neuropathic pain assessment

The Douleur Neuropathique en 4 questions (DN4) also known as the Neuropathic Pain Diagnostic Questionnaire was used to identify neuropathic pain (Harifi et al., 2011, Spallone et al., 2012). The DN4 is comprised of 10 questions (7 symptoms and 3 signs) and a score ≥4 identifies neuropathic pain with high sensitivity (83%) and specificity (90%) (Unal-Cevik et al., 2010).

7.3.5 Statistical analysis

Variables were compared between groups using a t-test and chi-squared test for continuous and categorical data, respectively. Data are expressed as mean [standard deviation (SD)] of mean.

Univariate analysis by simple linear regression was applied to determine which variables are associated with B_{12} levels, VPT and DN4 as outcome measures. Multiple linear regression analysis was used to determine the association between B_{12} levels, VPT and DN4 after adjusting for confounding factors. Residual plots were used to determine for linearity, normality, constant variance and independence.

All analyses were performed using StatsDirect version 3.0. A two-tailed P value of <0.05 was considered significant.

7.4 Results

Age, systolic (SBP), BMI, HbA1c, triglycerides, HDL and B_{12} levels were comparable between metformin (n=235) and non-metformin users (n=64). Metformin users had a shorter duration of diabetes (10.27 years ± 7.45 vs 12.89 years ± 8.89, *P*=0.03), but higher diastolic blood pressure (DBP) (77.72 mmHg ± 9.91 vs 74.52 mmHg ± 9.42, *P*=0.02), total cholesterol (4.48 mmol/L ± 1.10 vs 4.15 ± 1.02 mmol/L, *P*=0.03) and LDL (2.56 ± 0.88 mmol/L vs 2.30 ± 0.82 mmol/L, *P*=0.04). B_{12} levels were comparable between metformin and non-metformin users (*P*=0.87). However, the prevalence of B_{12} deficiency was lower in metformin (8%) compared to non-metformin (19%) users, P<0.01.

The prevalence of neuropathy (30% vs 39%) and neuropathic pain (31% vs 33%) were comparable between metformin and non-metformin users, respectively. The proportion of patients taking medications, which could influence B_{12} levels, including proton pump inhibitors (PPI), calcium supplements, multivitamins, B_{12} supplements and sulfonylureas were comparable between metformin and non-metformin users (Table 7.1).

Of the 362 T2D patients, 32 (8.8%) fulfilled the criteria for B_{12} deficiency (serum B_{12} <133 pmol/l). However, in those with B_{12} deficiency, the percentage taking metformin was significantly lower than in those without B_{12} deficiency (60% vs 80%, *P*=0.03). Patients with B_{12} deficiency were significantly younger (49.16 years ± 9.72 vs 54.56 years ± 12.71, *P* =0.01) and had a shorter duration of diabetes (7.03 years ± 5.39 vs 11.68 years ± 7.89, *P* <0.001), but comparable SBP, DBP, BMI, HbA1c, cholesterol, triglycerides, HDL and LDL. The prevalence and severity of neuropathy and neuropathic pain was comparable between those with and without B_{12} deficiency (Table 7.2).

Table 7.1. Comparison of demographic and clinical characteristics between non-metformin users and metformin users.

	Non-metformin users (n= 64)		Metformin users (n= 235)		P value
Demographics					
Age (years)	52.67	(13.95)	54.19	(11.61)	0.43
Diabetes duration (years)	12.89	(8.89)	10.27	(7.45)	0.03
SBP (mmHg)	128.88	(18.37)	130.23	(18.72)	0.61
DBP (mmHg)	74.52	(9.42)	77.72	(9.91)	0.02
BMI (kg/m^2)	31.79	(7.47)	32.10	(7.70)	0.78
HbA1c (%)	8.41	(2.22)	7.86	(1.89)	0.07
Total Cholesterol (mmol/l)	4.15	(1.02)	4.48	(1.10)	0.03
Triglycerides (mmol/l)	1.67	(1.12)	1.82	(1.14)	0.35
HDL (mmol/l)	1.15	(0.52)	1.05	(0.29)	0.18
LDL (mmol/l)	2.30	(0.82)	2.56	(0.88)	0.04
B_{12} levels					
B_{12} deficiency (<133 pmol/l) (%)	19		8		<0.01
B_{12} (pmol/l)	337.80	(280.34)	331.24	(247.61)	0.87
Medications					
Protein pump inhibitor (%)	45.8		42.9		0.81
Calcium supplements (%)	19.4		10.5		0.09
Multivitamins supplements (%)	14.5		14.5		0.99
Vitamin B supplements (%)	30.6		33.8		0.76
Sulfonylurea (%)	29.4		37.9		0.26
Neuropathy assessments					
Diabetic peripheral neuropathy (%)	39		30		0.21
Vibration perception threshold (V)	14.75	(12.15)	12.22	(9.07)	0.30
Neuropathic pain (%)	33		31		0.91
DN4	3.13	(3.08)	2.89	(2.63)	0.58

Data are presented as mean SD unless otherwise stated. Unpaired t- and $\chi 2$ test were applied to compare continuous and categorical data, respectively between the groups. Abbreviations: SBP=systolic blood pressure, DBP=diastolic blood pressure, VPT=vibration perception threshold, DN4=Neuropathic Pain Diagnostic Questionnaire.

Table 7.2. Comparison of demographic and clinical characteristics between those with (serum B_{12} <133 pmol/l) and without B_{12} deficiency. Data are presented as mean SD. Unpaired t- and X^2 test were used to compare continuous and categorical data, respectively between the groups. Abbreviations: SBP=systolic blood pressure, DBP=diastolic blood pressure, VPT=vibration perception threshold, DN4=Neuropathic Pain Diagnostic Questionnaire.

	B_{12} deficiency				
	Yes (n= 32)		No (n= 330)		P value
Demographics					
Metformin (%)	60		80		0.03
Age (years)	49.16	(9.72)	54.56	(12.71)	0.01
Diabetes duration (years)	7.03	(5.39)	11.68	(7.89)	<0.001
SBP (mmHg)	127.42	(15.47)	131.22	(19.58)	0.21
DBP (mmHg)	77.93	(11.32)	76.95	(9.72)	0.66
BMI (kg/m^2)	31.39	(6.48)	32.16	(7.60)	0.55
HbA1c (%)	7.76	(1.97)	7.99	(1.92)	0.52
Total Cholesterol (mmol/l)	4.30	(1.04)	4.47	(1.14)	0.39
Triglycerides (mmol/l)	1.53	(0.91)	1.77	(1.07)	0.19
HDL (mmol/l)	1.07	(0.41)	1.08	(0.34)	0.94
LDL (mmol/l)	2.61	(0.84)	2.49	(0.87)	0.49
Neuropathy assessments					
DPN (%)	32		33		0.85
VPT (V)	11.87	(9.51)	12.65	(9.19)	0.62
Neuropathic pain (%)	31		32		0.79
DN4	2.47	(2.98)	3.04	(2.59)	0.27

7.4.1 Association between vibration perception threshold, DN4 score and B_{12}

Simple linear regression analysis showed that VPT was positively associated with B_{12} (r=0.18 *P*<0.001). However, multiple linear regression analysis showed that this association was lost (β=0.003, *P*=0.25) after adjustment for confounding factors including, age, diabetes duration, SBP, HbA1c, and PPI use. DN4 had no association with B_{12} levels.

Simple linear regression analysis shows that B_{12} levels were not associated with the use of metformin, sulfonylurea or calcium supplementation, but were associated with age (r=0.15, *P*<0.01), duration of diabetes (r=0.16, *P*<0.01), HbA1c (r=0.11, *P*=0.05), vitamin D (r=0.17, *P*<0.01), PPI use (r=0.11, *P*<0.05), multivitamin use (r=0.11, *P*<0.05), and B_{12} supplementation (r=0.13, *P*<0.05). However, multiple linear regression analysis showed that B_{12} levels

maintained an association only with HbA1c (β=12.72, *P*=0.04) and vitamin D use (β=2.72, *P*=0.02), after adjustment for confounding factors.

7.5 Discussion

This is the first study from the Middle East region to assess the association between metformin exposure and B_{12} levels and its relationship to diabetic neuropathy. We show no difference in B_{12} levels between metformin and non-metformin users and actually show that the prevalence of B_{12} deficiency was lower in patients on metformin. This is in contrast to some but not all previously published studies (Chapman et al., 2016). Furthermore, we show no difference in the prevalence of diabetic peripheral neuropathy or painful diabetic neuropathy in T2D patients with and without B_{12} deficiency.

The 2018 ADA Clinical Practice Recommendations endorse screening metformin users for vitamin B_{12} deficiency (American Diabetes, 2018) and the 2017 ADA diabetic neuropathy statement recommends that all patients with diabetic neuropathy should be assessed for B_{12} deficiency, to exclude a treatable cause of neuropathy (Pop-Busui et al., 2017). However, previous studies examining the relationship between metformin use and B_{12} deficiency (Chapman et al., 2016); and indeed between B_{12} deficiency and neuropathy have been conflicting (Singh et al., 2013, Roy et al., 2016, Khan et al., 2017, Russo et al., 2016, Ahmed et al., 2016, Ma et al., 2015). Indeed, a study has shown a lower prevalence of DPN in T2D patients on metformin compared to those not on metformin (de Groot-Kamphuis et al., 2013). Marwan *et al* (2016) used the Neuropathy Total Scoring System (NTSS) and showed that subjects with normal B_{12} levels had a comparable prevalence of DPN to those with low B_{12} levels (36.8% vs 32.3%), and no correlation between B_{12} levels and NTSS (Ahmed et al., 2016). Russo *et al*. compared 79 subjects with DPN and 184 without DPN and found no relationship to metformin use (Russo et al., 2016). Chen *et al*. using a Neurothesiometer and monofilaments in addition to a structured questionnaire also showed no relationship between metformin use and peripheral neuropathy (Chen et al., 2012). In contrast Singh *et al*. showed that metformin users had lower levels of B_{12} and a higher Toronto Neuropathy Scoring System (Singh et al., 2013). Roy *et al*. (2016) showed that patients on metformin had a lower level of B_{12} and a reduction in Median, Ulnar and Peroneal nerve conduction (Roy et al., 2016). In the DPPOS study, whilst metformin was associated with an increased risk of B_{12}

deficiency, only 13 of the 56 participants on metformin with low vitamin B_{12} had neuropathy, but there was no difference in neuropathy symptoms or the total Michigan Neuropathy Screening Instrument score (Aroda et al., 2016). A recent study from India has shown an association between metformin use and B_{12} levels as well as DPN assessed using the Toronto Clinical Scoring System and median, ulnar, peroneal and posterior tibial nerve conduction velocity (Gupta et al., 2017).

Given that we showed a lower prevalence of B_{12} deficiency in patients taking metformin, we assessed confounding factors such as other medications, which may alter B_{12} levels. Sulphonylurea use in combination with metformin is a significant independent risk factor for B_{12} deficiency (Kang et al., 2014). B_{12} levels have also been reported to be lower in older adults with prolonged PPI and H2 blocker use in one study (den Elzen et al., 2008) but not in another study (Dharmarajan et al., 2008). Vitamin B supplementation is prevalent in the Middle East and may also influence B_{12} levels (El-Khateeb et al., 2014, Asiri and Al-Arifi, 2011). We show no association between B_{12} levels and concomitant use of sulphonylureas or calcium supplementations, but we do show small but significant associations with age, duration of diabetes, HbA1c and treatment with vitamin D, PPI's, multivitamins and B_{12}.

This is the first study to assess the relationship between metformin use B_{12} deficiency and the prevalence and severity of DPN and diabetic painful neuropathy in Qatar. The prevalence of both sensory neuropathy and painful diabetic neuropathy was comparable to previously published data (Malik et al., 2017, Almuhannadi et al., 2018, Petropoulos et al., 2016). A limitation of the present study is that it is a retrospective cohort study, but VPT and DN4 were assessed without the investigators being aware of the treatment or B_{12} status. The majority of patients had been prescribed metformin as first line therapy in accord with international guidelines, unless they were intolerant or it was withdrawn (American Diabetes, 2018), and therefore it was not possible to recruit a larger number of patients not on metformin. We cannot establish the exact duration of metformin exposure, although we can assume that metformin was prescribed shortly after diagnosis and therefore exposure is approximately equivalent to the duration of diabetes, which was approximately 10 years. As noted in the meta-analysis of Chapman et al the B_{12} lowering effect of metformin occurs within 6 weeks to 3 months of commencing metformin (Chapman et al., 2016).In future studies, it will be useful

to include methylmalonic acid (MMA) test to detect mild or early B12 deficiency. It will also be useful to account for other cofounders including dietary intake, ranitidine use and pernicious anemia to assess the association between metformin use and B_{12} deficiency.

In conclusion, we show no difference in B_{12} levels or the severity of DPN or painful diabetic neuropathy in metformin compared to non-metformin users. We also show no difference in vibration perception or painful diabetic neuropathy in those with and without B_{12} deficiency. These data urge the need for further larger, prospective studies to confirm or refute the current findings to support or challenge the highly prevalent practice of prescribing B_{12} for neuropathy across the Middle East.

7.6 Acknowledgements:

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Chapter 8: Effect of treatment with exenatide and pioglitazone or basal-bolus insulin on diabetic neuropathy: a substudy of the Qatar Study

Authors: Ponirakis G, Abdul-Ghani MA, Jayyousi A, Almuhannadi H, Petropoulos IN, Khan A, Gad H, Migahid O, Megahed A, DeFronzo R, Mahfoud Z, Hassan M, Al Hamad H, Ramadan M, Alam U, Malik RA. **BMJ Open Diabetes Research Care**. 31 August 2020, Issue 08. **DOI**: 10.1136/bmjdrc-2020-001420

8.1 Abstract

Introduction and objectives: To assess the effect of exenatide and pioglitazone or basal-bolus insulin on changes in diabetic peripheral neuropathy (DPN) measures in patients with poorly controlled type 2 diabetes (T2D).

Methods: This is a sub-study of the Qatar Study, an open label, randomized controlled trial. 38 subjects with poorly controlled T2D were studied at baseline and 1-year follow-up and 18 control subjects were assessed at baseline only. A combination of exenatide (2 mg/week) and pioglitazone (30 mg/day) or glargine with Aspart insulin were randomly assigned to patients to achieve an HbA1c <53 mmol/mol (<7%). DPN was assessed with CCM, DN4, vibration perception and sudomotor function.

Results: Subjects with T2D had reduced corneal nerve fiber density (CNFD), length (CNFL), and branch density (CNBD) but other DPN measures were comparable with the control group. In the combination treatment arm (n=21), HbA1c decreased by 35.2 mmol/mol (3.8 %) (P<0.0001), body weight increased by 5.6 Kg (P<0.0001), CNBD increased (P<0.05), vibration perception worsened (P<0.05), DN4 and sudomotor function showed no change. In the insulin treatment arm, HbA1c decreased by 28.7 mmol/mol (2.7 %) (P<0.0001), body weight increased by 4.6 Kg (P<0.01), CNBD and CNFL increased (P≤0.01), vibration perception improved (P<0.01), DN4 and sudomotor function showed no change. There was no association between the change in CCM measures with change in HbA1c, weight or lipids.

Conclusions: Treatment with exenatide and pioglitazone or basal-bolus insulin results in corneal nerve regeneration, but no change in neuropathic symptoms or sudomotor function over 1 year.

Keywords: Diabetic neuropathy; exenatide; pioglitazone; insulin; corneal confocal microscopy; type 2 diabetes

8.2 Introduction

Diabetic peripheral neuropathy (DPN) affects approximately 50% of patients with diabetes leading to neuropathic pain, erectile dysfunction and foot ulcers and imposes a significant health and economic burden to both the patient and health care providers (Pop-Busui et al., 2017). Whilst intensive glycemic control can prevent the onset or delay progression of DPN in type 1 diabetes (T1D) (Diabetes et al., 1993), there are conflicting data in type 2 diabetes (T2D) (Ohkubo et al., 1995, Ismail-Beigi et al., 2010, Pop-Busui et al., 2013, Azad et al., 1999, Callaghan et al., 2012, Maranta et al., 2020). Other cardiovascular risk factors, such as obesity (Schlesinger et al., 2019), hypertension (Ponirakis et al., 2019b), and hyperlipidemia (Tesfaye et al., 2005) are independently associated with DPN in T2D. Indeed, treatment with angiotensin converting enzyme (ACE) inhibitors (Malik et al., 1998, Reja et al., 1995) or statins (Davis et al., 2008, Villegas-Rivera et al., 2015) may have a beneficial effect on DPN.

Both glucagon-like peptide 1 (GLP-1) receptor agonists (Kan et al., 2012, Himeno et al., 2011) and thiazolidinediones (TZDs) (Qiang et al., 1998, Pop-Busui et al., 2013, Yamagishi et al., 2008, Wiggin et al., 2008) produce a durable reduction in HbA1c (Abdul-Ghani et al., 2017). GLP-1 receptor agonists stimulate insulin secretion in response to hyperglycemia, delay gastric emptying leading to weight loss and inhibit hepatic glucose secretion. In preclinical studies, exendin-4, a GLP-1 receptor agonist prevented sensory (Kan et al., 2012) and motor nerve conduction slowing (Himeno et al., 2011) and a reduction in intraepidermal nerve fiber density (IENFD) in T1D mice. However, twice daily exenatide, showed no effect on DPN in patients with T2D (Jaiswal et al., 2015). TZDs are potent insulin sensitizers and improve β-cell function. TZDs prevent nerve conduction slowing (Qiang et al., 1998), maintain myelinated fiber density, and reduce macrophage infiltration in the sciatic nerve (Yamagishi et al., 2008).

TZDs have been shown to reduce the incidence of DPN in patients with T2D (Pop-Busui et al., 2013).

There are currently no European Medicines Agency (EMA) and FDA approved therapies for DPN, despite multiple clinical trials. It has been suggested that the endpoints in these trials may not be sufficiently sensitive to detect a change in DPN (Malik, 2016). Several studies have provided support for the prevailing hypothesis that early subclinical small fiber injury precedes large fiber damage in DPN (Malik et al., 2011, Breiner et al., 2014). In this study, CCM was utilised to assess early small nerve fiber repair. Several longitudinal studies have shown that a lower corneal nerve fiber length (CNFL) at baseline predicts those patients who develop DPN (Pritchard et al., 2015, Lovblom et al., 2015, Edwards et al., 2017). CCM has also been used to identify early small fiber repair in several small clinical trials (Brines et al., 2015, Petropoulos et al., 2019). Indeed, CCM identified early corneal nerve regeneration 6 months after pancreas and kidney transplantation which was followed by an improvement in neuropathic symptoms and nerve conduction after 24 months (Tavakoli et al., 2013, Azmi et al., 2019b).

The Qatar Study (Abdul-Ghani et al., 2017) is an open-label, randomized controlled trial, which showed a rapid and effective reduction in HbA1c after treatment with a combination of exenatide and pioglitazone or basal-bolus insulin in patients with poorly controlled T2D. This is a sub-study of the Qatar study designed to assess the effect of the two treatment arms on changes in DPN measures with CCM as the primary outcome measure and DN4, vibration perception threshold (VPT) and sudomotor function as secondary outcome measures. This study also evaluated the effect of the treatments on diabetic retinopathy.

8.3 Materials and methods

This is an exploratory prospective sub-study of the Qatar Study (Abdul-Ghani et al., 2017), an open-label, randomized controlled trial (clinicaltrials.gov identifier NCT02887625) designed to examine the efficacy of exenatide plus pioglitazone versus basal-bolus insulin in patients with poorly controlled T2D on metformin plus sulfonylurea. This sub-study was added as an amendment to the Qatar study nearer to the completion date for recruitment and hence, it was not registered in a publicly available clinical trial database. Subjects with T2D were

enrolled from the National Diabetes Center in Hamad General Hospital and studied at baseline and 1-year follow-up and control subjects without diabetes were enrolled from Rumailah Hospital and studied between October 2016 and November 2018.

This study obtained ethics approval by the Institutional Review Board of Hamad Medical Corporation (IRB#: 13-00076) and all participants gave informed consent before taking part in the study. The research adhered to the tenets of the declaration of Helsinki.

8.3.1 Study cohort

Subjects were eligible to participate if they were between 18-75 years old, had poorly controlled (HbA1c >58 mmol/mol [7.5%]) T2D treated with a maximal dose of metformin (>1,500 mg/day) plus sulfonylurea (>4 mg glimepiride or >60 mg gliclazide); normal kidney and liver function, electrocardiogram and stable body weight (±1 Kg within the preceding year). Healthy controls had a HbA1c <6%.

Exclusion criteria were any cause of neuropathy other than diabetes (chemotherapy, HIV infection, and hepatitis C), factors that may affect the corneal nerves (severe dry eyes, severe corneal dystrophies, ocular trauma or surgery in the preceding 6 months), a hematocrit <34%, medications known to affect glucose metabolism other than sulfonylureas and metformin, evidence of diabetic proliferative retinopathy, albumin excretion >300 mg/day, and major organ system disease, as determined by physical examination, medical history, and screening blood tests.

8.3.2 Interventions

In the Qatar study, eligible subjects were randomized to receive exenatide plus pioglitazone or glargine and aspart to achieve and maintain an HbA1c <53 mmol/mol (<7%). There was no limit on the upper value of HbA1c for enrolment. Subjects randomized to combination treatment were started on weekly subcutaneous extended release exenatide (2 mg/week Bydureon) and pioglitazone (30 mg/day). Subjects receiving insulin were started on glargine before breakfast. The Treat-to-Target Trial (4T) algorithm was used to calculate the starting glargine dose, and the dose was adjusted weekly to achieve a fasting plasma glucose (FPG) of <6.11 mmol/L. After the FPG goal was achieved, if the HbA1c was >53 mmol/mol (>7.0%), 4–

6 units of insulin aspart was started before each meal, and the dose was adjusted to achieve a post-prandial plasma glucose concentration of <7.78 mmol/L, 2 hours after meals. Patients were seen monthly during the first 4 months or as needed, based on the results of the plasma glucose concentration, and bimonthly thereafter. The percentage of subjects experiencing hypoglycemia during the 1-year trial was calculated as the number of subjects experiencing at least one single episode of hypoglycemia (blood glucose concentration <60 mg/dL with or without symptoms or hypoglycemic symptoms that subsided following glucose ingestion) divided by the number of patients in that arm as per the protocol in the Qatar study (Abdul-Ghani et al., 2017).

8.3.3 Diabetic neuropathy assessment

CCM was performed using a Heidelberg Retina Tomograph 3 with the Rostock Cornea Module (Heidelberg Engineering GmbH). The CCM utilizes a 670 nm diode laser and provides digital images of the cornea. The technique has been previously described (Petropoulos et al., 2013b). Briefly, both eyes were anesthetized using Oxybuprocaine hydrochloride 0.4% (Conjuncain EDO; Fabrik GmbH) followed by a drop of carbomer 0.2% eye gel (Blumont Healthcare Ltd.) and patients were instructed to fixate on a target. Several scans of the subbasal nerve plexus in the central cornea were captured per eye for 2 minutes. Adjacent images were separated by approximately 1-4 µm. CCM image extraction was carried out at a separate time by one investigator unaware of the treatment group. Three high clarity CCM images per eye were selected based on a previously published protocol (Petropoulos et al., 2013b) and images were selected based on depth, focus position and contrast (Kalteniece et al., 2017). Corneal nerve fiber density (CNFD) (fibers/mm^2), corneal nerve branch density (CNBD) (branches/mm^2), and corneal nerve fiber length (CNFL) (mm/mm^2) were quantified using CCMetrics, a validated image analysis software (Dabbah et al., 2011).

Vibration perception threshold (VPT) was measured using a Neurothesiometer (Horwell Scientific Laboratory Supplies) on the pulp of the large toe on both feet and the average value of three measurements was recorded as a VPT in Volts (V) ranging from 0-50V.

Sudomotor function was measured by electrochemical skin conductance (ESC) using Sudoscan (Impeto Medical SAS) as described previously. Sudoscan evaluates sympathetic

innervation based on sweat chloride concentrations generated by the sweat gland in response to the voltage applied and is reported as ESC in microSiemens (μS).

Neuropathic pain was assessed using the Douleur Neuropathique en 4 (DN4) questionnaire based on symptoms and signs as previously described (Azmi et al., 2019c).

8.3.4 Diabetic retinopathy assessment

Ophthalmic examination was carried using a non-contact slit-lamp biomicroscope (Topcon) with +90 D lens (Volk) and two digital retinal images of both eyes were taken using a digital fundus camera (Zeiss) after pupil dilatation with Tropicamide 1% in 16/21 patients in the combination treatment group and 9/17 patients in the insulin treatment group. Diabetic retinopathy was graded by two qualified investigators according to the NHS Diabetic Eye Screening Programme (Team, 2012). Diabetic retinopathy was graded as R0 for no diabetic retinopathy, R1 for the presence of microaneurysms, retinal hemorrhages, venous loops, exudates or cotton wool spots in the presence of other features of diabetic retinopathy and R2 for the presence of venous beading, reduplication, multiple blot hemorrhages or intraretinal microvascular abnormality. Diabetic proliferative retinopathy (R3) was an exclusion criterion. Maculopathy was defined as M0 for no maculopathy or for any microaneurysm or hemorrhage within 1 disc diameter of the center of the fovea if associated with a best VA of 6/12 where the cause of the reduced vision is known and is not diabetic macular edema and M1 for exudate, retinal thickening, microaneurysm or hemorrhage within 1 disc diameter of the center of the fovea or a group of exudates within the macula.

8.3.5 Outcome measures

The primary outcome measures were the CCM measures and the secondary outcome measures were DN4, VPT and sudomotor function.

8.3.6 Statistical analysis

Due to the small cohort, this sub-study was not adjusted for multiple comparisons (Rothman, 1990). The results were analysed as an exploratory study. Continuous variables between controls, subjects with T2D treated with exenatide plus pioglitazone and insulin were

compared using one-way ANOVA with Bonferroni's post hoc test for pairwise comparisons. Continuous variables between the two groups were compared using an unpaired t-test. Categorical variables were compared using x^2. Changes between baseline and 1-year follow-up were compared using a paired t-test. Correlation of the change in CCM measures with the change in HbA1c, body weight and lipids were analyzed using the Pearson correlation coefficient. All analyses were performed using IBM-SPSS (version 23; SPSS Inc, Armonk NY). A two-tailed P value of ≤0.05 was considered significant.

8.4 Results

8.4.1 Baseline characteristics

The exenatide plus pioglitazone (n=21) and insulin (n=17) group had comparable HbA1c (92.5±18.8 mmol/mol [10.6±1.7%] vs 89.9±22.5 mmol/mol [10.4±2.1%], P=0.7) and significantly higher than the control group (41.6±5.0 mmol/mol [6.0±0.5%], P<0.0001) (Table 8.1). The mean age, gender, lipid profile, diastolic blood pressure (DBP), body weight and BMI were comparable between all three groups. The systolic blood pressure (SBP) in the combination treatment group was significantly lower than in the control group (126.4 mmHg vs 143.7 mmHg, P<0.05) and insulin treatment group (130.8±19.3, P=0.02). The percentage of patients with diabetic retinopathy was comparable between the two treatment groups (31.3% vs 44.4%, P=0.51).

The combination treatment group had significantly lower corneal nerve fiber density (CNFD, fibers/mm^2) (26.1 vs 33.7, P=0.01), branch density (CNBD, branches/mm^2) (57.0 vs 110.4, P<0.001) and fiber length (CNFL, mm/mm^2) (17.8 vs 25.1, P=0.0001) compared to the control group. The insulin treatment group had significantly lower CNBD (70.3 branches/mm^2, P<0.01) and CNFL (19.4 mm/mm^2, P<0.01) compared to the control group. There was no difference in vibration perception threshold and sudomotor function measured by electrochemical skin conductance between the three groups and the percentage of patients with neuropathic pain (DN4 >4) was comparable between the treatment groups.

Table 8.1. Baseline characteristics of patients with type 2 diabetes who received exenatide plus pioglitazone or insulin treatment.

	Controls (n=18)	Exenatide plus pioglitazone (n=21)	Basal-bolus insulin (n=17)	P value
Age, years	53.0±11.0	50.1 ±9.4	54.9 ±7.5	0.30
Male, n (%)	13/18 (72.2)	11/21 (52.4)	12/17 (70.6)	0.35
Duration of diabetes, years	N/A	10.0 ±5.9	13.1 ±9.3	0.24
HbA1c, mmol/mol	41.6±5.0	92.5 ±18.8†††	89.9 ±22.5†††	<0.0001
HbA1c, %	6.0±0.5	10.6 ±1.7†††	10.4 ±2.1†††	
Total cholesterol, mmol/l	5.0±1.0	4.7 ±0.6	5.3 ±1.3	0.17
Triglyceride, mmol/l	1.4±0.5	2.0 ±1.4	1.8 ±1.0	0.37
HDL, mmol/l	1.2±0.3	1.3 ±0.6	1.2 ±0.4	0.87
LDL, mmol/l	3.2±0.9	2.6 ±0.7	3.1 ±1.0	0.10
Systolic BP, mmHg	143.7±12.7	126.4 ±17.9‡	130.8 ±19.3	0.02
Diastolic BP, mmHg	82.1±6.6	78.2 ±13.9	77.6 ±10.4	0.50
Body weight, Kg	75.8±4.7	87.9 ±19.5	84.3 ±13.7	0.07
BMI, Kg/m^2	28.8±3.4	32.4 ±6.7	30.4 ±5.9	0.2
Diabetic retinopathy, n (%)		5/16 (31.3)	4/9 (44.4)	0.51
Neuropathic pain, n (%)		4/18 (22.2)	2/12 (16.7)	0.71
CNFD, fibers/mm^2	33.7±5.7	26.1 ±7.9†	28.8 ±9.1	0.01
CNBD, branches/mm^2	110.4±45.0	57.0 ±31.6††	70.3 ±31.2†	<0.001
CNFL, mm/mm^2	25.1±4.3	17.8 ±4.9†††	19.4 ±5.7†	0.0001
VPT, V	7.2±4.1	7.3 ±4.6	11.4 ±7.4	0.08
ESC feet, µS	66.9±18.4	59.8 ±25.7	67.2 ±12.0	0.55

Numeric variables and frequency distribution for categorical variables are summarized as means ±standard deviation or n (%). Variables were compared using one-way ANOVA except for duration of diabetes which was compared using unpaired t-test. Categorical variables were compared using x^2. Variables that were significantly different between controls and patients with T2D were denoted as ‡P≤0.05, †P≤0.01, ††P≤0.001, †††P≤0.0001. Abbreviations: BP= blood pressure, CNFD=corneal nerve fiber density, CNBD=corneal nerve branch density, CNFL=corneal nerve fiber length, VPT=vibration perception threshold and ESC =electrochemical skin conductance.

8.4.2 Change in clinical and metabolic variables

HbA1c reduced significantly in both treatment groups (P<0.0001), more so with exenatide plus pioglitazone compared with basal-bolus insulin (35.2 mmol/mol [3.8%] vs 28.7 mmol/mol [2.7%], P<0.05) (Figure 8.1). The mean HbA1c at 1-year follow-up was lower but not significant in the combination treatment group (51.4±12.0 mmol/mol [6.9±1.1%] vs 60.2±18.2 mmol/mol [7.7±1.7%], P=0.1) (Table 8.2). A higher percentage of patients achieved the ADA treatment goal of HbA1c <53 mmol/mol (<7.0%) in the combination treatment group compared to the insulin treatment group (15/21 [71.4%] vs 6/17 [35.3%], P<0.05). The

percentage of patients with hypoglycemia in the insulin group was significantly higher than the combination group (84.6% vs 38.1%, P=0.008).

Body weight increased by 4.6 Kg in the insulin group and by 5.6 Kg in the combination treatment group (P<0.01) (Figure 8.2).

In both treatment groups, total cholesterol decreased by 0.5-0.8 mmol/l (P<0.05-0.001). In the combination treatment group, triglycerides decreased by 0.4 mmol/l (P<0.05) and diastolic blood pressure decreased by 8.9 mmHg (P<0.0001) and high-density lipoprotein cholesterol (LDL) increased by 0.4 mmol/l (P<0.01).

Figure 8.1. Effect of exenatide plus pioglitazone and insulin treatment on HbA1c over 1-year. Overall HbA1c changes between different time points across 12 months were compared using paired t-test: ‡P≤0.05, †P≤0.01, ††P≤0.001, †††P≤0.0001. Combinational therapy (n=21), insulin therapy (n=17). Error bars show standard deviation.

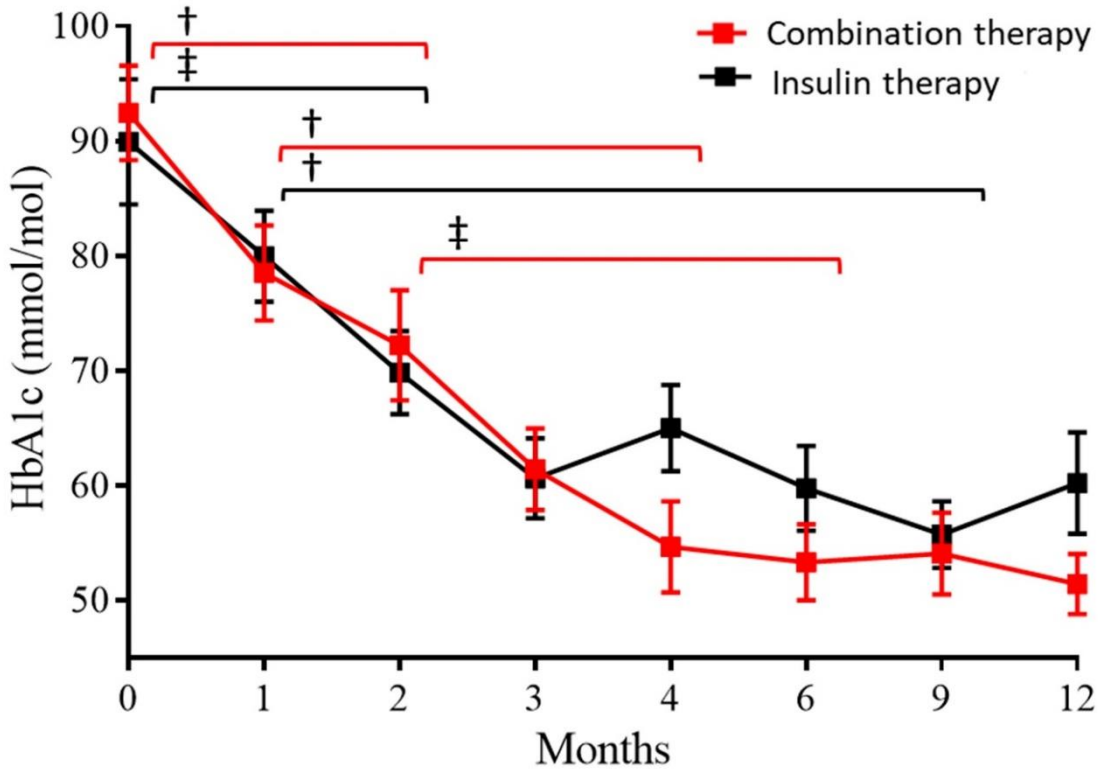


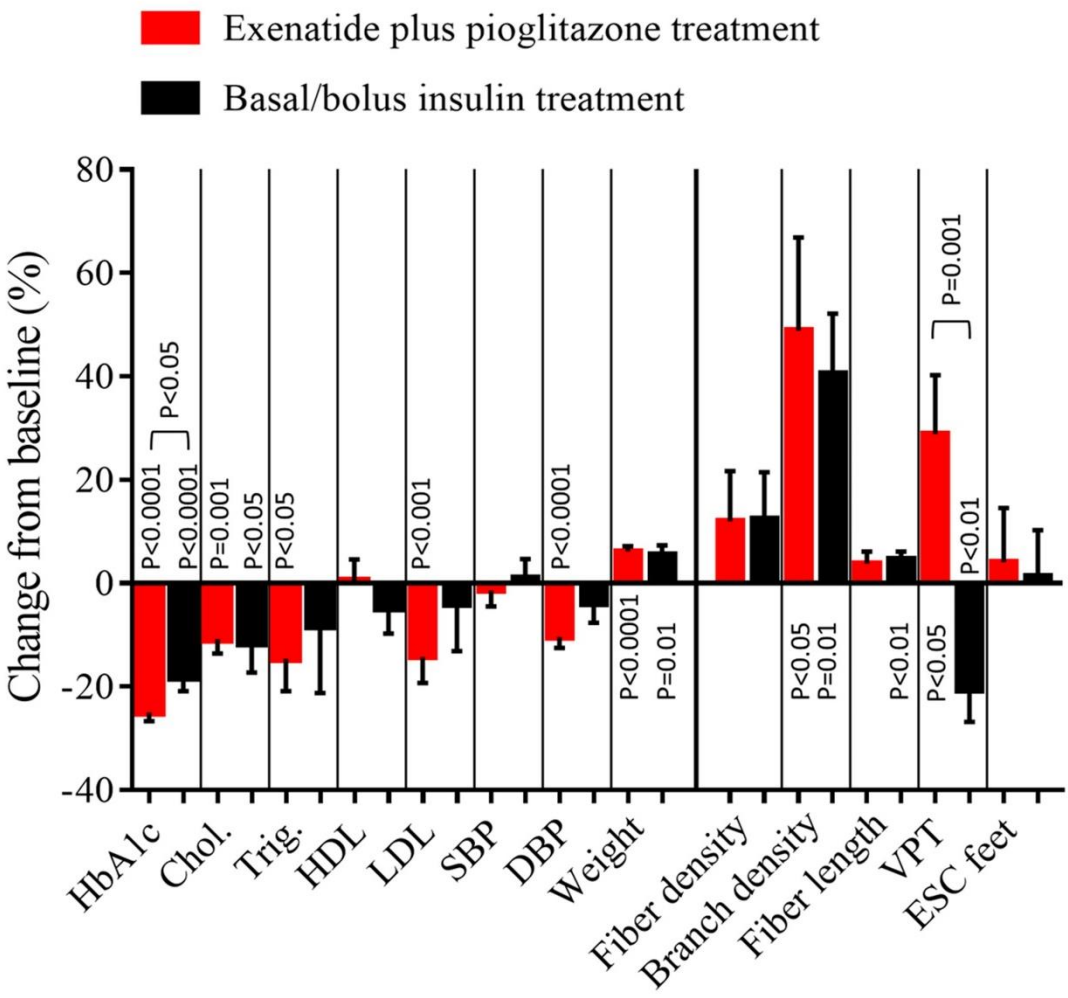
Table 8.2. Changes in clinical and metabolic variables and measures of DPN after 1-year of exenatide plus pioglitazone or insulin treatment.

	Exenatide plus pioglitazone (n=21)		Basal-bolus insulin (n=17)		P value[1]	P value[2]
	1-year follow-up	Change	1-year follow-up	Change		
HbA1c, mmol/mol	51.4 ±12.0	-35.2†††	60.2 ±18.2	-28.7†††	0.1	<0.05
Hb1Ac, %	6.9 ±1.1	-3.8†††	7.7 ±1.7	-2.7†††		
Total cholesterol, mmol/l	4.2 ±0.8	-0.5††	4.5 ±0.9	-0.8‡	0.28	0.40
Triglyceride, mmol/l	1.6 ±1.3	-0.4‡	1.4 ±0.7	-0.4	0.57	1.00
HDL, mmol/l	1.2 ±0.3	-0.1	1.1 ±0.2	-0.1	0.40	0.82
LDL, mmol/l	2.2 ±0.8	-0.4†	2.7 ±0.8	-0.3	0.06	0.92
Systolic BP, mmHg	123.4 ±16.8	-3.0	130.4 ±15.8	-0.4	0.20	0.65
Diastolic BP, mmHg	69.3 ±10.5	-8.9†††	73.9 ±10.6	-3.8	0.20	0.14
Body weight, Kg	93.5 ±22.0	5.6†††	88.9 ±15.8	4.6†	0.47	0.62
BMI, Kg/m^2	33.2 ±7.3	0.8‡	30.1 ±5.5	-0.3	0.17	0.09
Diabetic retinopathy, n (%)	13/16 (81.3)	8†	6/9 (66.7)	2	0.41	
Neuropathic pain, n (%)	2/18 (11.1)	-2	2/12 (16.7)	0	0.66	
CNFD, fibers/mm^2	26.6 ±5.3	0.6	30.8 ±8.9	2.0	0.11	0.61
CNBD, branches/mm^2	76.0 ±38.6	19.0‡	97.4 ±54.2	27.2†	0.20	0.51
CNFL, mm/mm^2	19.7 ±4.8	1.9	21.7 ±5.8	2.3†	0.28	0.79
VPT, V	9.0 ±5.4	1.7‡	8.7 ±5.9	-2.8†	0.87	0.001
ESC feet µS	61.8 ±23.4	2.0	65.5 ±15.3	-1.7	0.65	0.53

P value[1] for combination vs insulin therapy at 1-year follow-up
P value[2] for combination vs insulin therapy changes at 1-year follow-up

Numeric variables and frequency distribution for categorical variables are summarized as means ±standard deviation or n (%). Continuous variable between exenatide plus pioglitazone and insulin treatment were compared using unpaired t-test. Categorical variables were compared using x^2. Changes between baseline and 1-year follow-up were compared using paired t-test: ‡P≤0.05, †P≤0.01, ††P≤0.001, †††P≤0.0001. Abbreviations: BP= blood pressure, CNFD=corneal nerve fiber density, CNBD=corneal nerve branch density, CNFL=corneal nerve fiber length, VPT=vibration perception threshold and ESC =electrochemical skin conductance.

Figure 8.2. Effect of exenatide plus pioglitazone and insulin treatment on HbA1c, lipid profile, blood pressure, body weight, corneal nerve fiber measures, vibration perception threshold and sudomotor function in the feet over 1-year follow-up. Abbreviations: VPT=vibration perception threshold and ESC =electrochemical skin conductance. Combination treatment (n=21) and basal/bolus insulin treatment (n=17). Error bars show standard deviation.



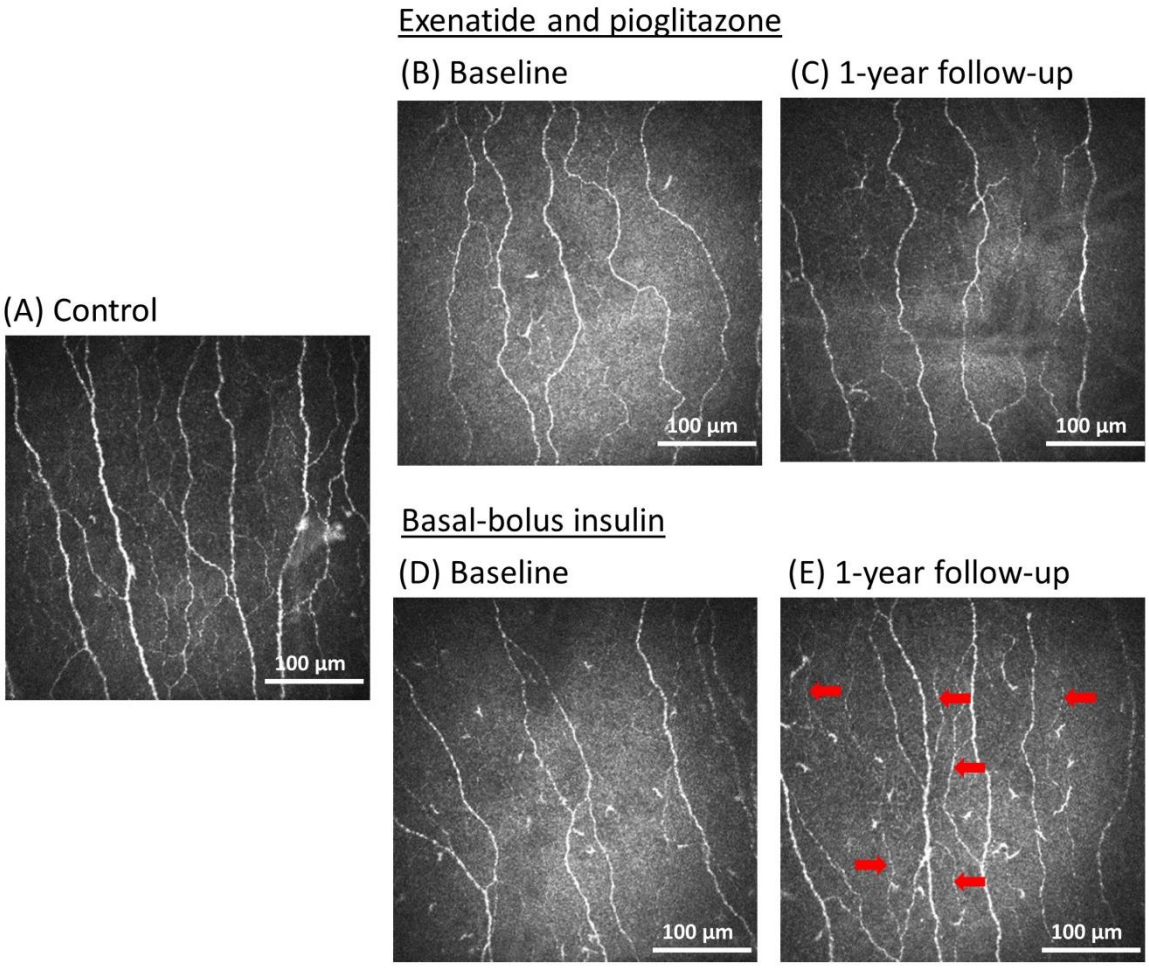
8.4.3 Change in neuropathy measures

In the insulin treatment group, CNBD and CNFL increased by 27.2 branches/mm^2 (P=0.01) and 2.3 mm/mm^2 (P<0.01), respectively, with no change in CNFD (Figure 8.2 and 8.3). In the exenatide plus pioglitazone treatment group, CNBD increased by 19.0 branches/mm^2 (P=0.02) with no change in CNFD (P=0.76) and CNFL (P=0.12). Between the treatment groups the change in CNFD (26.6 fibers/mm^2 vs 30.8 fibers/mm^2, P=0.11), CNBD (76.0 branches/mm^2 vs 97.4 branches/mm^2, P=0.20) and CNFL (19.7 mm/mm^2 vs 21.7 mm/mm^2, P=0.28) were comparable.

Vibration perception threshold decreased by 2.8 V (P<0.01) in the insulin treatment group and increased by 1.7 V (P<0.05) in the combination treatment group, with a significant difference between the two treatment groups at 1-year follow-up (P=0.001) (Table 8.2 and Figure 8.2).

There was no significant change in the percentage of patients with neuropathic pain (DN4 >4). There was no change in sudomotor function in either treatment group (Table 8.2).

Figure 8.3. Corneal confocal microscopy (CCM) images of the sub-basal nerve plexus. Corneal nerve morphology in: (A) healthy age-matched controls, people with T2D treated with exenatide and pioglitazone (B & C) and basal-bolus insulin (D & E) at baseline and 1-year follow-up. The red arrows indicate the fibers that might have increased the measurement of nerve branches and fiber length in the insulin treatment group at 1-year follow-up.



8.4.4 Correlation between change in CCM measures with change in Hb1Ac, lipids and weight

There was no correlation between the percentage change in CNFD with percentage change in HbA1c (r=0.06, P=0.74), total cholesterol (r=0.16, P=0.37), triglycerides (r=0.20, P=0.25), HDL (r=0.08, P=0.66) and weight (r=0.24, P=0.17). There was no correlation between percentage change in CNBD with percentage change in HbA1c (r=0.01, P=0.95), total cholesterol (r=0.06, P=0.74), triglycerides (r=0.08, P=0.64), HDL (r=0.06, P=0.73) and weight (r=0.25, P=0.14). There was no correlation between percentage change in CNFL with percentage change in HbA1c (r=0.05, P=0.77), total cholesterol (r=0.12, P=0.49), triglycerides (r=0.14, P=0.42), HDL (r=0.04, P=0.82) and weight (r=0.03, P=0.84)

8.4.5 Diabetic retinopathy

The percentage of patients with new onset diabetic retinopathy increased significantly from 31.3% to 81.3% (P<0.01) in the combination treatment group and whilst there was an increase in the insulin treatment group from 44.4% to 66.7%, this was not significant (Table 8.2). Eight subjects in the combination treatment group and two subjects in the insulin group progressed from R0 to R1. There was no progression of retinopathy in subjects graded R1, R2, M0 or M1 at baseline.

8.5 Discussion

This study shows that combination treatment with exenatide plus pioglitazone or basal-bolus insulin over 12 months results in a marked improvement in HbA1c, but with weight gain, and hypoglycemia, consistent with the Qatar study (Abdul-Ghani et al., 2017). Insulin treatment was associated with a significant improvement in distal corneal nerve morphology characterised by an increase in corneal nerve branch density and length and an improvement in vibration perception, but no change in sudomotor function or incidence of neuropathic pain. Combination treatment was associated with an improvement in the lipid profile, blood pressure and an increase in distal corneal nerve branch density, but a small but significant deterioration in VPT with no change in sudomotor function or incidence of neuropathic pain. The improvement in CCM measures were independent of changes in HbA1c, body weight and

lipids. There was an increase in the incidence of diabetic retinopathy in the combination treatment group.

Whilst exenatide results in weight loss (Jaiswal et al., 2015), pioglitazone is associated with weight gain, explaining the increase in weight observed in the combination treatment group. Obesity (Schlesinger et al., 2019) is a risk factor for DPN. Jaiswal et al. (Jaiswal et al., 2015) reported that exenatide resulted in 3 kg weight loss after 1-year, compared with 2 kg weight gain with glargine (Ismail-Beigi et al., 2010). Pioglitazone is associated with a lowering of diastolic blood pressure and triglycerides and we also observed a significant reduction in diastolic blood pressure and triglycerides in the combination treatment group. Hypertension (Ponirakis et al., 2019b) and hyperlipidemia (Tesfaye et al., 2005) are also risk factors for DPN. However, the weight gain in both treatment arms may have limited the overall benefit on neuropathy.

Glucagon-like peptide 1 (GLP-1) receptor agonists have been reported to have a neuroprotective effect. In preclinical studies, Himeno *et al.* (Himeno et al., 2011) showed that exendin-4 prevented both sensory and motor nerve conduction slowing and reduction of IENFD. However, Kan *et al*. (Kan et al., 2012) reported that exendin-4 prevented sensory nerve conduction slowing but had no effect on motor nerve conduction slowing and epidermal innervation. Conversely, in T2D mice, exendin-4 prevented motor nerve conduction slowing but had no effect on sensory nerve conduction. In a clinical trial of patients with T2D treated with exenatide there was no effect on the incidence of DPN, cardiovascular autonomic neuropathy (CAN) or IENFD over 18 months (Jaiswal et al., 2015). Recently, the LEADER trial (Dhatariya et al., 2018) showed that liraglutide was associated with a significantly lower risk of amputations related to diabetic foot ulceration in patients with T2D. However, a study of 39 patients with T1D and established neuropathy randomized to liraglutide or placebo over 26 weeks recently failed to show a benefit on autonomic function or sensory and motor nerve conduction (Brock et al., 2019). Thiazolidinediones (TZDs) have also been reported to have a neuroprotective effect. In preclinical studies, Qiang *et al*. (Qiang et al., 1998) reported that troglitazone prevented nerve conduction slowing and maintained normal myelinated fiber architecture and density in T1D rats. Yamagishi *et al*. (Yamagishi et al., 2008) confirmed that pioglitazone prevented nerve conduction slowing and reduced macrophage infiltration in the

sciatic nerve in T1D rats. Wiggin *et al*. (Wiggin et al., 2008) showed that rosiglitazone prevented thermal hypoalgesia and reduced oxidative stress in the sciatic nerve of T1D mice. In the BARI 2D trial (Pop-Busui et al., 2013), rosiglitazone significantly reduced the 4-year cumulative incidence of DPN compared to insulin treatment. The neuroprotective effect of TZDs may be attributed to a reduction in oxidative stress and advanced glycated end products. Our data suggest that exenatide plus pioglitazone treatment may be associated with small fiber regeneration, assessed using CCM.

In preclinical studies, Kan *et al*. (Kan et al., 2012) reported that high-dose insulin prevented a reduction of IENFD in T1D mice but had no effect in T2D mice. In the DCCT, intensive insulin treatment reduced the incidence of clinical DPN by 60% (Diabetes et al., 1993) and prevented peroneal nerve conduction velocity slowing over a 5-year period in patients with T1D. However, in patients with T2D the UKPDS (UK Prospective Diabetes Study (UKPDS) Group, 1998) and VA-CSDM trial (Azad et al., 1999) reported that intensive treatment had no effect on the incidence of DPN and CAN compared with conventional treatment. The Kumamoto study (Ohkubo et al., 1995) showed that intensive treatment prevented nerve conduction slowing over 6 years and the ACCORD trial (Ismail-Beigi et al., 2010) showed a reduction in the incidence of loss of ankle reflexes but no effect on VPT over 6-years (Callaghan et al., 2012). Our data suggest that insulin treatment might have a beneficial effect on DPN, independent of the improvement in glycemic control as there was evidence of greater small nerve fiber regeneration and an improvement in vibration perception. In a previous study comparing continuous subcutaneous insulin infusion (CSII) with multiple daily insulin injection (MDI) we showed that despite a comparable HbA1c, the CSII group showed an increase in CNFD, CNBD and CNFL (Azmi et al., 2015), which was attributed to a direct neurotrophic effect of insulin (Guo et al., 2011).

Both combination and insulin treatment improved corneal nerve fiber measures but had no effect on neuropathic symptoms or sudomotor function over 1 year. This is consistent with studies showing corneal nerve regeneration 6 months after pancreas and kidney transplantation in T1D with no change in quantitative sensory testing and an improvement in neuropathic symptoms and nerve conduction at 24 and 36 months (Mehra et al., 2007, Tavakoli et al., 2013, Azmi et al., 2019b). Autonomic function has not been shown to improve

3, 8 and 10 years after kidney and pancreas transplantation (Azmi et al., 2019b, Navarro et al., 1997, Havrdova et al., 2016), but multifactorial risk factor reduction showed an improvement in cardiac autonomic function with no change in vibration perception threshold (Gaede et al., 2008). A recent study from Japan showed that multifactorial risk factor reduction achieved by improving and even normalizing glycemic control and reducing body weight and blood pressure in patients with T2D over 4 years, resulted in an improvement in CNFL, CNBD, neurophysiology and vibration perception, which correlated with the reduction in HbA1c (Ishibashi et al., 2019). The present study shows an improvement in CNBD and CNFL, but no change in sudomotor function over 12 months. Jaiswal et al. (Jaiswal et al., 2015) reported a trend for a greater increase in IENFD 1-year after capsaicin denervation in patients on insulin compared to exenatide. In a randomized placebo-controlled trial of once weekly C-peptide there was no improvement in sural nerve conduction velocity or the modified Toronto Clinical Neuropathy Score and yet vibration perception threshold improved significantly (Wahren et al., 2016). These findings emphasize the importance of the type and duration of intervention and choice of end points in clinical trials of DPN.

A large improvement in HbA1c (>2-3%) has been reported to be associated with treatment-induced neuropathic pain, autonomic neuropathy and a worsening of retinopathy and microalbuminuria (Gibbons and Freeman, 2015). Our study shows that despite a reduction in HbA1c of 3.8% with a combination of exenatide and pioglitazone and 2.7% with insulin, there was no increase in the incidence of painful DPN. However, the genesis of painful neuropathy is complex and may involve alterations in transient receptor potential channels, which may not have been altered by the current interventions (Roa-Coria et al., 2019). The incidence of diabetic retinopathy increased, especially in the combination treatment group. GLP-1 therapy has been associated with an increase in the risk of retinopathy progression in patients with diabetic retinopathy in a large randomized trial with semaglutide (Marso et al., 2016), although two large population based analyses have failed to confirm this association (Douros et al., 2018, Pfeffer et al., 2015). Treatment with lixisenatide and once weekly exenatide have previously shown no adverse effect on retinopathy (Pfeffer et al., 2015).

We acknowledge this is a small open-label study with a lack of blinding for participants and investigators due to weekly exenatide injections and multiple daily insulin injections.

However, the PhD student that evaluated the neuropathy outcome measures was masked to the treatment group. Our cohort of patients with T2D had minimal neuropathy and a very effective reduction in HbA1c over 12 months leading to early small nerve fiber repair as observed after simultaneous pancreas-kidney (SPK) transplantation (Azmi et al., 2019b) or optimal medical therapy (Ishibashi et al., 2019).

In conclusion, exenatide plus pioglitazone or basal-bolus insulin treatment effectively reduces HbA1c and promotes small fiber regeneration. Whilst the incidence of diabetic retinopathy increased, especially in the combination treatment group, there was no impact on neuropathic pain. Our findings support the utility of CCM as an early surrogate marker of therapeutic response in clinical trials of diabetic neuropathy.

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Chapter 9: Association of corneal nerve fiber measures with cognitive function in dementia

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9.1 Abstract

Introduction: CCM is a non-invasive ophthalmic technique that identifies corneal nerve degeneration in a range of peripheral neuropathies and in patients with multiple sclerosis, Parkinson's disease and amyotrophic lateral sclerosis.

Objectives: We sought to determine whether there is any association of corneal nerve fiber measures with cognitive function and functional independence in patients with MCI and dementia.

Methods: In this study, 76 non-diabetic participants with MCI (n=30), dementia (n=26) and healthy age-matched controls (n=20) underwent assessment of cognitive and physical function and CCM.

Results: There was a progressive reduction in corneal nerve fiber density (CNFD), branch density (CNBD) and fiber length (CNFL) ($P<0.0001$) in patients with MCI and dementia compared to healthy controls. Adjusted for confounders, all three corneal nerve fiber measures were significantly associated with cognitive function ($P<0.05$) and functional independence ($P<0.01$) in MCI and dementia. The area under the ROC curve to distinguish MCI with CNFD, CNBD and CNFL was 69.1%, 73.2% and 73.0% and for dementia it was 84.8%, 84.2% and 86.2%, respectively.

Conclusions: CCM demonstrates corneal nerve fiber loss, which is associated with a decline in cognitive function and functional independence in patients with MCI and dementia.

9.2 Introduction

Dementia is a progressive neurodegenerative disease, which currently affects 47 million people world-wide (Prince et al., 2015). It is a cause of significant cognitive and functional disability, and is the most common cause of death in women over 80 years of age in the UK (Morgan and Rutty, 2016). Neurodegeneration underlies accelerated cognitive decline and can be identified by brain atrophy (Leung et al., 2013, Eskildsen et al., 2013, McDade et al., 2018), hypometabolism (Landau et al., 2010, Herholz, 2010) and hypoperfusion (Metastasio et al., 2006). Neurodegeneration can be detected approximately 15 years before overt cognitive decline associated with Alzheimer's disease (AD).(McDade et al., 2018) The National Institute of Aging and the Alzheimer's Association (NIA-AA) have emphasized the need for biomarkers of neurodegeneration to identify those at greatest risk for cognitive decline or progression from mild cognitive impairment (MCI) to dementia (Albert et al., 2011, Sperling et al., 2011).

There is an increasing focus on identifying markers for neurodegeneration, which can detect pre-clinical disease especially for disease modifying or preventative strategies (Cummings, 2017). There is good evidence that the neurodegenerative process in AD is not limited to the brain but also occurs in the retina as a thinner retinal nerve fiber layer (RNFL) is associated with cognitive decline in patients with MCI and AD (Ko et al., 2018, Khawaja et al., 2016, Shi et al., 2014). Corneal confocal microscopy (CCM) is a non-invasive ophthalmic imaging technique which allows quantification of corneal nerve morphology and may act as a potential marker for neurodegeneration. It has been most extensively used to study patients with diabetic neuropathy (Malik et al., 2003, Quattrini et al., 2007b, Perkins et al., 2018) and other peripheral neuropathies including those associated with CIDP (Stettner et al., 2016), HIV (Kemp et al., 2017), Fabry disease (Bitirgen et al., 2018) and inherited neuropathies such as CMT1A (Tavakoli et al., 2012) and Friedreich's ataxia (Pagovich et al., 2018). However, more recent studies have shown that CCM can also identify nerve fiber loss in patients with Parkinson's disease (Kass-Iliyya et al., 2015, Podgorny et al., 2016), amyotrophic lateral sclerosis (Ferrari et al., 2014), and multiple sclerosis (Bitirgen et al., 2017a, Petropoulos et al., 2017, Bitirgen et al., 2017b, Mikolajczak et al., 2016).

The objectives of this study were to: (1) determine whether there is significant corneal nerve fiber loss in patients with MCI and dementia compared to age-matched controls and (2) determine the association between corneal nerve fiber measurements with cognitive function and functional independence.

9.3 Methods

Patients with mild cognitive impairment (MCI), dementia and healthy age-matched controls were recruited from the Geriatric clinic in Rumailah Hospital, Doha, Qatar between September 2016 and May 2018. Patients with severe anxiety, depression, Parkinson's disease, frontotemporal and Lewy body dementia, hypomania and severe dementia who were unable to cooperate were excluded. Furthermore, patients with systemic diseases that may affect corneal nerve fibers, including diabetes, vitamin B12 deficiency, hypothyroidism, HIV infection and hepatitis C, were excluded. In addition, patients with dry eyes, corneal dystrophies, ocular trauma or surgery in the preceding 6 months were also excluded. We enrolled 222 people and excluded 117 patients with diabetes, 1 patient with depression, 1 patient with hypomania, 3 people younger than the inclusion age and 24 people who did not complete the assessments to leave a sample size of 76. This study was approved by the Institutional Review Board (IRB) of Weill Cornell Medicine in Qatar (WCM-Q) and Hamad Medical Corporation (HMC) and all participants gave informed consent to take part in the study. The research adhered to the tenets of the declaration of Helsinki.

9.3.1 Demographic and metabolic measures

Data including age, ethnicity, gender, blood pressure, weight, and body mass index (BMI) were recorded. HbA1c, lipids, creatinine, hemoglobin (Hgb), mean corpuscular volume (MCV), serum vitamin B_{12}, vitamin D, free thyroxine (FT4) and thyroid stimulating hormone (TSH) were assessed.

9.3.2 Cognitive screening

Cognitive screening was administered by the occupational therapist using the Montreal cognitive assessment (MoCA) Arabic and English version. The MoCA is a 30 point test and includes seven cognitive domains: visuospatial abilities (clock-drawing, cube copy, and

alternation task adapted from the Trail-Making B task), naming (confrontation naming of 3 animals), attention (including the sum of attention, concentration, and working memory items), language (the sum of repetition of sentences and verbal fluency task scores), abstract thinking/executive functions (the 2-item verbal abstraction), short-term memory/recall, and orientation. MoCA scores below 26 were considered to indicate cognitive impairment (Nasreddine et al., 2012). A point was added for individuals who had formal education ≤6th grade. Patients with cognitive symptoms of depression were determined based on clinical interview and were excluded from the study. Cognitive symptom duration was estimated from the clinical history obtained from relatives and participants.

9.3.3 Functional Independence assessment

The Functional Independence Measure (FIM) was administered by the occupational therapist and is an 18-point screening test of which 13 are for motor and 5 for cognitive function and each point is scored from 1 to 7. The total FIM score ranges from 18 to 126. There is no cut-off point for FIM, but a higher score indicates greater independence (Tanaka et al., 2013).

9.3.4 Diagnosis

The diagnosis of MCI and dementia were based on the NIA-AA guideline (McKhann et al., 2011) and the Diagnostic and Statistical Manual 4th edition (DSM IV) diagnostic criteria (Trull et al., 2012). A joint consultative model in the Department of Geriatric Medicine run by geriatricians and geriatric psychiatrists with advice and consultation from the neurologists was applied to ensure the correct diagnosis, especially to exclude reversible, complex and young-onset dementia. The diagnosis of MCI or dementia was based on a comprehensive history and examination, which includes 1) presenting complaint and history of illness; 2) comprehensive history of each of the cognitive domains; 3) psychiatric history for ruling out depression, mood disorders and psychosis; 4) medical history including episodes of delirium and other medical comorbidities; 5) medication history; 6) functional history of basic daily living activities; 7) components of comprehensive geriatric assessment; 8) detailed psychiatric mental status examination and cognitive screening using MoCA. Subsequent analysis included comprehensive organic work-up which are blood investigation and brain imaging. It is through this robust diagnostic process that the psychiatrists applied the diagnostic criteria. The final

diagnosis (control, MCI, dementia) was made according to consensus decision. Radiological evidence for Alzheimer's disease (AD), included volume loss of hippocampi, entorhinal cortex, and amygdala on MRI, based on the criteria described by Dubois et al. (Dubois et al., 2009) For vascular dementia, the NINDS-AIREN criteria (Roman et al., 1993) which specify evidence of cerebrovascular disease by brain imaging (MRI) were applied and includes multiple large vessel infarcts or a single strategically placed infarct (angular gyrus, thalamus, basal forebrain, or posterior (PCA) or anterior cerebral artery (ACA) territories), multiple basal ganglia and white matter lacunes, extensive periventricular white matter lesions, or combinations thereof. The neuroradiologists also looked for potentially reversible causes of cognitive decline such as tumors, subdural hematoma or normal pressure hydrocephalus.

9.3.5 Corneal Confocal Microscopy

Participants underwent corneal confocal microscopy (CCM), a non-invasive ophthalmic imaging technique using the Heidelberg Retina Tomograph and the Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany) (Petropoulos et al., 2013d, Petropoulos et al., 2013b). The patient's eyes were anesthetized using a drop of 0.4% benoxinate hydrochloride, and Viscotears were applied on the front of the eye for lubrication. A drop of Viscotears was placed between the tip of the objective lens and a sterile disposable TomoCap allowing optical coupling of the objective lens to the cornea. The patient was instructed to fixate on a target with the eye not being examined. Several scans of the sub-basal nerve plexus in the central cornea were captured per eye for ~2 minutes. The field of view of each image is 400X400 μm. At a separate time, three high clarity images per eye were selected by one researcher blind to the patient diagnosis. Criteria for image selection were depth, focus position and contrast (Kalteniece et al., 2017). Three corneal measures: corneal nerve fiber density (CNFD) (number of main nerve fibers/mm^2), branch density (CNBD) (number of branches/mm^2), and fiber length (CNFL) (length of main nerves and branches mm/mm^2) were quantified manually using CCMetrics, a validated image analysis software (Dabbah et al., 2011).

9.3.6 Statistical analysis

The sample size required to determine a significant difference in corneal nerve fiber measures between the control, MCI, and dementia group was calculated from our previously published data (Chen et al., 2015). Given a reported difference in population means of 8 no./mm^2 for CNFD, with an estimated standard deviation of 7, we estimated that ~17 participants for each group would be needed to provide a study power of 80% and an alpha of 0.05.

Patients' demographic and clinical characteristics were summarized using means and standard deviations for numeric variables and frequency distribution for categorical variables. Variables were compared between the controls; MCI and dementia group using one-way analysis of variance (ANOVA) with Bonferroni's post hoc test for pairwise comparisons and Chi-square test, respectively. Correlation analysis between the three corneal nerve fiber measures was performed using Pearson's method.

Univariate analysis by simple linear regression was performed with age, gender, systolic and diastolic blood pressure, weight, BMI, HbA1c, cholesterol, triglyceride, HDL, LDL, Hgb, MCV, TSH, FT4, vitamin B_{12}, cognitive function, duration of cognitive impairment, functional independence, MCI and dementia as independent variables, and the corneal nerve fiber measures as the dependent variable. The multiple linear regression analysis included all variables with P≤0.05 at the bivariate level. The regression *coefficient (beta) and* the corresponding *95% confidence intervals* (*95% CI*) are presented. Residual plots were used to determine for linearity, normality, constant variance, and independence.

Receiver operating characteristic (ROC) curve analysis was used to determine the ability of CNFD, CNBD and CNFL to distinguish patients with MCI and dementia from healthy controls. The area under curve (AUC), and two cut-off point with the maximal sum of sensitivity and specificity was calculated.

All analyses were performed using IBM-SPSS (version 23; SPSS Inc, Armonk NY). Dot plots were generated using GraphPad Prism, version 6.05. A two-tailed P value of ≤0.05 was considered significant.

9.4 Results

9.4.1 Demographic and clinical characteristics

The demographic and clinical characteristics are summarized in Table 9.1. Participants (n=76) with mild cognitive impairment (MCI) (n=30) and dementia (n=26) were compared with a control group (n=20). The groups had comparable age, gender, systolic blood pressure (SBP), weight, body mass index (BMI), HbA1c, triglycerides, high density lipoprotein (HDL), creatinine, hemoglobin (Hgb) and mean corpuscular volume (MCV). The dementia group had a significantly lower diastolic blood pressure compared to the MCI group ($P<0.05$), a lower cholesterol than both the control and MCI group ($P<0.05$) and lower low-density lipoprotein (LDL) compared to the control group ($P<0.05$). More patients with dementia were on a statin (n=12, 46%) compared to controls (n=4, 20%), which may explain the lower total cholesterol in the dementia group. There was a progressive reduction in cognitive function measured by the Montreal Cognitive Assessment (MoCA) between the control (27.30 ± 4.21), MCI (24.04 ± 2.93, $P<0.05$) and dementia group (12.96 ± 5.65, $P<0.0001$). The duration of cognitive impairment was significantly longer in the dementia (3.35 ±3.07 years) compared to the MCI (1.48 ± 1.66 years, $P<0.01$) group. The Functional Independence Measure (FIM) was lower in the dementia group (84.80 ± 29.01) compared to the MCI (120.9 ± 6.5, $P<0.0001$) and control (125.23 ± 1.30, $P<0.0001$) group, but did not differ between the control and MCI group. The dementia group consisted of participants with Alzheimer's disease (n=7, 27%), vascular dementia (n=6, 23%) and mixed dementia (n=13, 50%).The study cohort was comprised of 16 (21.1%) Qatari Arabs, 30 (39.5%) other Arabs, 21 (27.6%) South Asians, 7 (9.2%) Africans and 2 (2.6%) Caucasians.

9.4.2 Corneal nerve fiber measures

The corneal nerve fiber morphology and measures in patients with MCI and dementia, and healthy age-matched controls are shown in Figure 9.1. The MCI group compared to the control group had a significantly lower corneal nerve branch density (CNBD) ($P<0.01$) and corneal nerve fiber length (CNFL) ($P<0.05$), with no significant difference in the corneal nerve fiber density (CNFD). CNBD, CNFL and CNFD ($P<0.0001$) were all significantly reduced in the dementia group compared to the control group and CNFD ($P<0.01$) and CNFL ($P<0.05$) were significantly lower in the dementia group compared to the MCI group. All three corneal nerve fiber measures were significantly correlated to each other; CNFD with CNBD ($r= 0.70$, $P<0.0001$) and CNFL ($r= 0.70$, $P<0.0001$) and CNBD with CNFL ($r= 0.92$, $P<0.0001$).

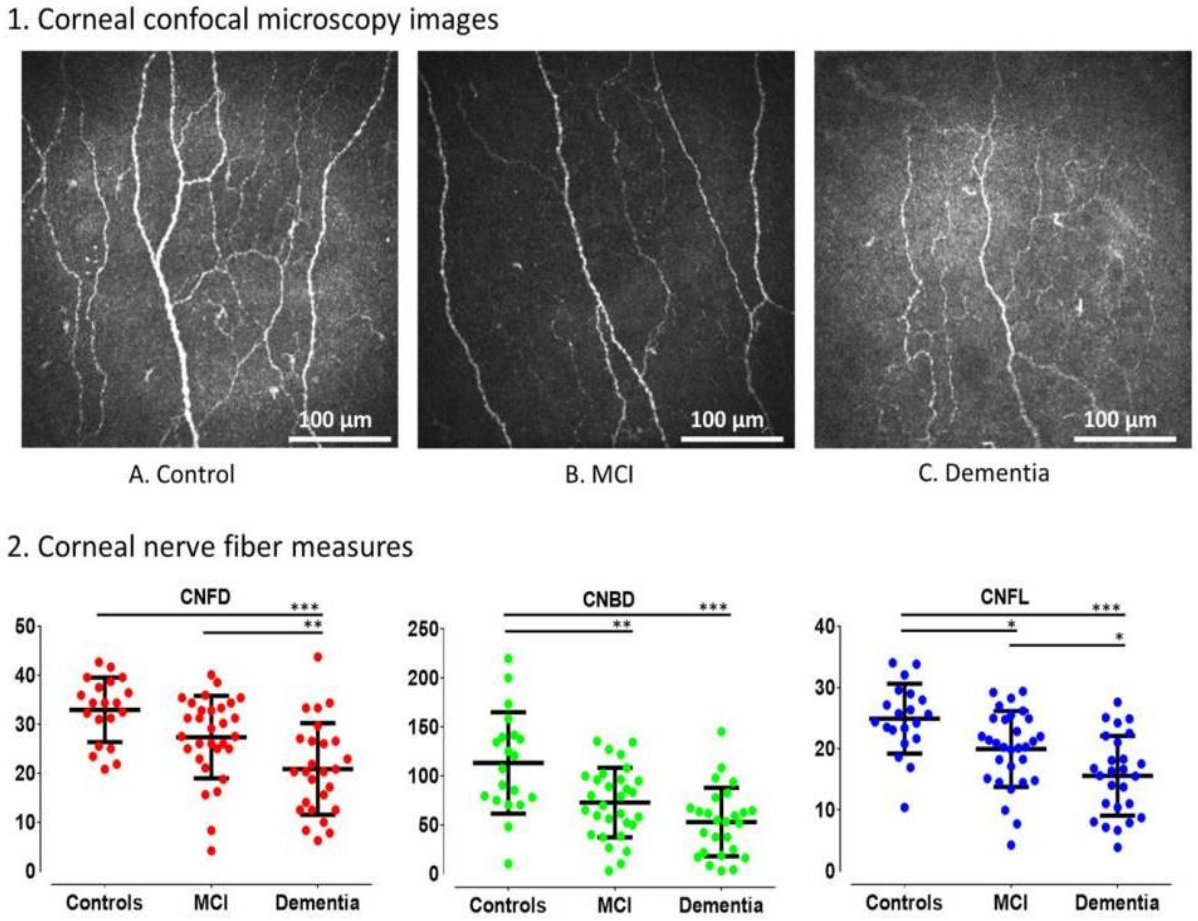
Table 9.1. Demographic and clinical characteristics of the study population.

	Controls (n= 20)	MCI (n= 30)	Dementia (n= 26)	P value[1]	P value[2]	P value[3]
Demographics						
Age, mean ± SD, years	67.65±9.02	67.83±8.48	72.62±8.53	NS	NS	NS
Gender, n (%) Male	14 (28.6)	19 (38.8)	16 (32.7)	NS	NS	NS
Female	6 (22.2)	11 (40.7)	10 (37.0)			
BP sys, mean ± SD, mmHg	137.75±11.39	140.62±14.20	138.35±24.95	NS	NS	NS
BP dias, mean ± SD, mmHg	76.85±10.86	76.97±6.59	70.56±10.37	NS	NS	<0.05
Weight, mean ± SD, Kg	73.30±8.74	80.78±18.61	76.61±12.90	NS	NS	NS
BMI, mean ± SD, Kg/m^2	27.39±3.06	35.12±24.68	30.14±5.32	NS	NS	NS
HbA1c, mean ± SD, %	5.74±0.41	5.64±0.59	5.61±0.42	NS	NS	NS
Chol. mean ± SD, mmol/l	5.11±0.95	4.96±0.89	4.24±1.10	NS	<0.05	<0.05
Trig. mean ± SD, mmol/l	1.27±0.53	1.28±0.63	1.39±0.68	NS	NS	NS
HDL mean ± SD, mmol/l	1.34±0.37	1.34±0.54	1.27±0.47	NS	NS	NS
LDL mean ± SD, mmol/l	3.18±0.86	2.98±0.83	2.36±0.94	NS	<0.05	NS
Creatinine mean ± SD, µmol/l	82.10±25.39	79.79±27.20	82.75±28.28	NS	NS	NS
Hgb, mean ± SD, gm/dL	14.11±1.65	13.30±1.84	13.28±1.01	NS	NS	NS
MCV, mean ± SD, fL	88.41±5.28	82.59±10.52	86.69±5.90	NS	NS	NS
Cognitive function						
MoCA, mean ± SD	27.30±4.21	24.04±2.93	12.96±5.65	<0.05	<0.0001	<0.0001
Cognitive impairment duration, mean ± SD, years	0±0	1.48±1.66	3.35±3.07	0.05	<0.0001	<0.01
Physical and social function						
FIM, mean ± SD	125.23±1.30	120.9±6.5	84.80±29.01	NS	<0.0001	<0.0001
Corneal nerve fiber measures						
CNFD, mean ± SD, no./mm^2	32.95±6.60	27.38±8.42	20.88±9.36	NS	<0.0001	<0.01
CNBD, mean ± SD, no./mm^2	113.29±51.76	72.83±35.62	52.91±34.88	<0.01	<0.0001	NS
CNFL, mean ± SD, mm/mm^2	24.93±5.70	19.97±6.21	15.58±6.51	<0.05	<0.0001	<0.05

[1] Control vs MCI
[2] Control vs dementia
[3] MCI vs dementia

Characteristics of 76 participants presented as mean ± standard deviation for numeric variables and frequency distribution for categorical variables for healthy age-matched controls, people with mild cognitive impairment (MCI) and dementia. Continuous and categorical variables were compared using one-way ANOVA with Bonferroni's post hoc test and Chi-square test, respectively. Abbreviations: MoCA=Montreal cognitive assessment, FIM=Functional independence measure, CNFD=corneal nerve fiber density, CNBD=corneal nerve branch density and CNFL=corneal nerve fiber length.

Figure 9.1. Corneal nerve fiber morphology and measures in healthy age-matched controls, people with mild cognitive impairment (MCI) and dementia.



(1) Corneal confocal microscopy (CCM) images of the sub-basal nerve plexus in (A) a 70 year-old control showing normal corneal nerve fiber morphology; (B) a 69 year old patient with MCI and (C) a 69 year old patient with dementia showing a progressive reduction in corneal nerve fiber density, branch density and length. (2) Dot plots of corneal nerve fiber density (CNFD) (red), corneal nerve branch density (CNBD) (green) and corneal nerve fiber length (CNFL) (blue) in controls, people with MCI and dementia. The line that extends from the middle of the vertical line represents the mean and the lines that extend to the top and bottom are the standard deviation with significant differences between the control, MCI and dementia group (*P≤0.05, **P≤0.01, ***P<0.0001).

9.4.3 Association of corneal nerve fiber measures with cognitive function, duration of cognitive impairment and functional independence in MCI and dementia

Univariate analysis with CNFD and CNBD as dependent variables showed a significant association with cognitive function (β=0.41 and 0.39, P≤0.01), duration of cognitive impairment (β= -0.32 and -0.30, P<0.05), functional independence (β=0.52 and 0.45, P≤0.01), MCI (β= -0.30 and -0.28, P≤0.05), dementia (β= -0.59 and -0.58, P<0.0001) and total cholesterol (β=0.26 and 0.25, P≤0.05). Univariate analysis with CNFL as a dependent variable showed a significant association with cognitive function (β=0.42, P<0.0001), duration of cognitive impairment (β= -0.30, P<0.01), functional independence (β=0.54, P<0.0001), MCI (β= -0.27, P=0.05), dementia (β= -0.61, P<0.0001), age (β= -0.23, P=0.05) and total cholesterol (β=0.29, P≤0.05).

Multiple linear regression analyses to determine the association of corneal nerve fiber measures with cognitive function, functional independence, MCI, dementia and duration of cognitive impairment are summarised in Table 9.2. Adjusted for cholesterol, CNFD and CNBD were associated with cognitive function (β=0.31, 0.33, P<0.05), functional independence (β=0.50, 0.67, P<0.01) and dementia (β= -0.48, -0.55, P<0.01), but only CNBD was associated with MCI (β= -0.38, P<0.01). Adjusted for age and cholesterol, CNFL was associated with cognitive function (β=0.31, P<0.05), functional independence (β=0.56, P=0.001), MCI (β= -0.33, P<0.05) and dementia (β= -0.51, P<0.01). However, the association of corneal nerve fiber measures with duration of cognitive impairment was lost after adjusting for confounding factors.

9.4.4 CCM sensitivity and specificity

The AUC for MCI with CNFD, CNBD and CNFL was 69.1% (95% CI, 53.7% - 84.4%), 73.2% (95% CI, 58.6% - 87.9%) and 73.0% (95% CI, 58.7% - 87.3%), respectively and for dementia it was 84.8% (95% CI, 73.6% - 96.0%), 84.2% (95% CI, 72.2% - 96.3%) and 86.2% (95% CI, 75.5% - 96.9%), respectively (Figure 9.2). Using a CNFD cut-off of <34 no./mm^2, the sensitivity for MCI and dementia was 76.7% and 92.3%, respectively and the specificity was 55%. Using a CNBD cut-off of <78 no./mm^2, the sensitivity for MCI and dementia was 53.3% and 80.8%, and the specificity was 70% and 75%, respectively. Using a CNFL cut-off of <23 mm/mm^2 CNFL, the

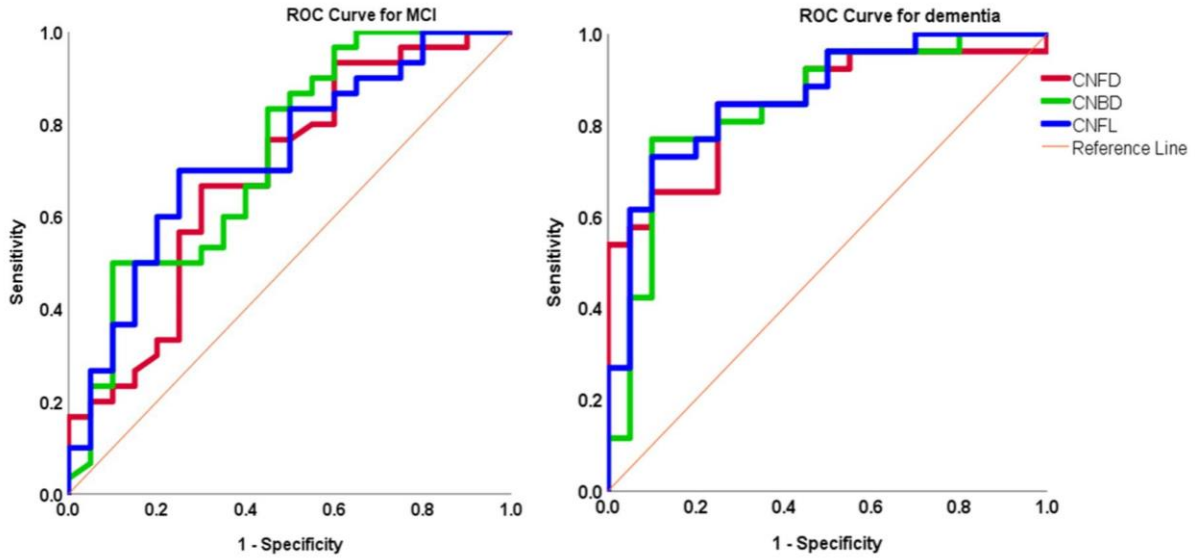
sensitivity for MCI and dementia was 70.0% and 84.6%, respectively and the specificity was 75%.

Table 9.2. Multiple linear regression analysis to determine the association of corneal nerve fiber measures with cognitive function, functional independence, mild cognitive impairment (MCI), dementia and duration of cognitive impairment.

	Coefficient	95% Confidence Interval	P value
Montreal Cognitive assessment (MoCA)			
CNFD, no./mm^2	0.31	0.06, 0.80	<0.05
CNBD, no./mm^2	0.33	0.54, 4.87	0.01
CNFL, mm/mm^2	0.31	0.04, 0.66	<0.05
Function Independence Measure (FIM)			
CNFD, no./mm^2	0.67	0.16, 0.38	<0.0001
CNBD, no./mm^2	0.50	0.46, 2.08	<0.01
CNFL, mm/mm^2	0.56	0.08, 0.31	0.001
Mild cognitive impairment (MCI)			
CNFD, no./mm^2	-0.27	-8.21, 0.28	NS
CNBD, no./mm^2	-0.38	-61.08, -9.24	<0.01
CNFL, mm/mm^2	-0.33	-7.67, -0.37	<0.05
Dementia			
CNFD, no./mm^2	-0.48	-7.57, -1.66	<0.01
CNBD, no./mm^2	-0.55	-45.64, -12.50	0.001
CNFL, mm/mm^2	-0.51	-6.20, -1.45	<0.01
Duration of cognitive impairment			
CNFD, no./mm^2	-0.24	-2.19, 0.08	NS
CNBD, no./mm^2	-0.24	-12.76, 0.54	NS
CNFL, mm/mm^2	-0.23	-1.74, 0.10	NS

The following confounding variables were considered: cholesterol for CNFD and CNBD, and age and cholesterol for CNFL. All the variables considered in the fitted model had P<0.05. Abbreviations: CNFD=corneal nerve fiber density, CNBD=corneal nerve branch density and CNFL=corneal nerve fiber length.

Figure 9.2. ROC analysis showing the area under the curve for CCM measures in distinguishing people with MCI and dementia from healthy controls. The area under the ROC curve to distinguish MCI with CNFD, CNBD and CNFL was 69.1%, 73.2% and 73.0% and for dementia it was 84.8%, 84.2% and 86.2%, respectively.



9.5 Discussion

This study shows that CCM detects corneal nerve fiber loss in people with mild cognitive impairment (MCI) and people with dementia, compared to age-matched healthy controls. Furthermore, after adjusting for confounding factors, corneal nerve fiber loss was significantly associated with decline in cognitive function and functional independence in patients with MCI and dementia. This is an important observation as it demonstrates cognitive decline is not only associated with brain atrophy (Leung et al., 2013, Eskildsen et al., 2013) and retinal nerve fiber layer (RNFL) thinning (Ko et al., 2018, Khawaja et al., 2016, Shi et al., 2014), but also with corneal nerve fiber loss.

The diagnosis of MCI and dementia are based on clinical, cognitive, and functional criteria as well as clinical judgment (Albert et al., 2011). However, there is no sharp demarcation between aging cognition and MCI and between MCI and dementia. The NIA-AA proposed a classification scheme for preclinical AD based on biomarkers of β-amyloid, tauopathy and neurodegeneration to determine the level of certainty for progression from MCI to Alzheimer's disease (AD) (Albert et al., 2011, Sperling et al., 2011). Current NIA-AA recommended markers for neurodegeneration include brain atrophy (Leung et al., 2013, Eskildsen et al., 2013, McDade et al., 2018), hypometabolism (Landau et al., 2010, Herholz,

2010) and hypoperfusion (Metastasio et al., 2006) using magnetic resonance imaging (MRI), PET, and single-photon emission computed tomography (SPECT) imaging, respectively. However, the clinical utility of these biomarkers is hampered by the invasiveness of cerebrospinal fluid (CSF) sampling and high costs or limited availability of MRI, PET, and SPECT (Albert et al., 2011, McKhann et al., 2011).

There are several studies suggesting that the eye may be a biomarker for dementia (Ko et al., 2018, Khawaja et al., 2016, Misra et al., 2017). The European Prospective Investigation of Cancer study of 8,623 people in the UK showed that RNFL thinning was associated with cognitive decline (Khawaja et al., 2016). Similarly, in 32,038 healthy UK Biobank participants RNFL thinning was associated with future cognitive decline (Ko et al., 2018). A recent study in patients with Parkinson's disease has shown that a reduction in corneal nerve fiber length was associated with cognitive function as assessed using the Addenbrooke's cognitive examination-revised (ACE-R) score (Misra et al., 2017). There are no prior published data examining the association between corneal nerve morphology and cognitive function in people with MCI or dementia. In the present study, the diagnostic workup employed the Arabic and English version of the Montreal cognitive assessment (MoCA), which is considered to be a good index of cognitive impairment compared to the Mini-Mental State Examination (MMSE), especially for MCI (Nasreddine et al., 2005). All three corneal nerve fiber measures were associated with a decline in cognitive function and functional independence. The ROC curve analysis suggests that CCM may have a good discriminative power to distinguish between healthy people and people with dementia. Paradoxically, we show that patients with a lower CNFL have a lower total cholesterol, which is counter to previous studies showing that corneal nerve fiber loss is associated with increased levels of cholesterol (Alamri et al., 2019, Andersen et al., 2018). However, this may be explained by the 2-fold greater use of statins in patients with dementia.

The association between corneal nerve fiber loss and cognitive function should be interpreted with caution, especially with the small cohorts studied. Sub-analysis to assess any difference in the corneal nerve fiber measurements for Alzheimer's disease and vascular dementia will be undertaken in future larger cohort studies. We acknowledge, there may be other causes of corneal nerve fiber loss such as impaired glucose tolerance and metabolic syndrome,

although we carefully excluded participants with ocular diseases, corneal dystrophies, diabetes and other causes of neuropathy that may influence corneal nerves. Nevertheless, this study suggests corneal confocal microscopy can identify neurodegeneration in people with MCI and dementia and is associated with cognitive decline and functional independence. In the present study the diagnostic workup employed was detailed and we used the Arabic and English version of the Montreal cognitive assessment (MoCA), which is considered to be a good index of cognitive impairment compared to the Mini-Mental State Examination (MMSE), especially for MCI (Nasreddine et al., 2005). Larger, longitudinal studies are required to establish the diagnostic and prognostic utility of CCM in people with MCI and dementia.

9.6 Acknowledgements:

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Chapter 10: Corneal nerve and brain imaging in mild cognitive impairment and dementia

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10.1 Abstract

Introduction: Visual rating of medial temporal lobe atrophy (MTA) is an accepted structural neuroimaging marker of Alzheimer's disease. Corneal confocal microscopy (CCM) is a non-invasive ophthalmic technique that detects neuronal loss in peripheral and central neurodegenerative disorders.

Objectives: To determine the diagnostic accuracy of CCM for mild cognitive impairment (MCI) and dementia compared to medial temporal lobe atrophy (MTA) rating on MRI.

Methods: Subjects aged 60-85 with no cognitive impairment (NCI), MCI and dementia based on the ICD-10 criteria were recruited. Subjects underwent cognitive screening, CCM and MTA rating on MRI.

Results: 182 subjects with NCI (n=36), MCI (n=80) and dementia (n=66), including AD (n=19, 28.8%), VaD (n=13, 19.7%) and mixed AD (n=34, 51.5%) were studied. CCM showed a progressive reduction in corneal nerve fiber density (CNFD, fibers/mm^2) (32.0$\pm$7.5 vs 24.5$\pm$9.6 vs 20.8$\pm$9.3, p<0.0001), branch density (CNBD, branches/mm^2) (90.9$\pm$46.5 vs 59.3$\pm$35.7 vs 53.9$\pm$38.7, p<0.0001) and fiber length (CNFL, mm/mm^2) (22.9$\pm$6.1 vs 17.2$\pm$6.5 vs 15.8$\pm$7.4, p<0.0001) in subjects with MCI and dementia compared to NCI. The area under the ROC curve (95% CI) for the diagnostic accuracy of CNFD, CNBD, CNFL compared to MTA-right and MTA-left for MCI was 78% (67-90%), 82% (72-92%), 86% (77-95%) vs 53% (36-69%) and 40% (25-

55%), respectively, and for dementia it was 85% (76-94%), 84% (75-93%), 85% (76-94%) vs 86% (76-96%) and 82% (72-92%), respectively.

Conclusions: The diagnostic accuracy of CCM, a non-invasive ophthalmic biomarker of neurodegeneration was high and comparable with MTA rating for dementia but was superior to MTA rating for MCI.

10.2 Introduction

Dementia is a progressive neurodegenerative disease affecting 40-50 million people worldwide (Wu et al., 2017, Prince et al., 2013). Therapeutic and psychological interventions for people with early stage dementia can improve cognition, independence, and quality of life (Prince et al., 2011). However, the clinical diagnosis of mild cognitive impairment (MCI) or early dementia is challenging due to the insidious onset of disease and gradual cognitive decline. The National Institute on Aging and the Alzheimer's Association (NIA-AA) has proposed a number of biomarkers that reflect the underlying pathology of the disease to support the diagnosis of MCI and dementia (Albert et al., 2011, McKhann et al., 2011).

Medial temporal lobe atrophy (MTA) rating is an established biomarker for neurodegeneration in Alzheimer's disease (AD) but not for MCI or dementia (Albert et al., 2011, McKhann et al., 2011). There is progressive MTA in subjects with MCI and dementia compared to those with no cognitive impairment (NCI) (Du et al., 2001, Urs et al., 2009). MTA rating has been shown to have high diagnostic accuracy for probable (Thies et al., 1999) and established AD (Heo et al., 2013, Cavedo et al., 2014). It can distinguish subjects with and without amnesic MCI and predict transition from NCI to MCI and from MCI to probable AD (Duara et al., 2008) as well as cognitive decline (Velickaite et al., 2018). MTA has also been reported in patients with vascular dementia (VaD) (Barber et al., 2000, Cho et al., 2009).

CCM is a rapid non-invasive ophthalmic imaging technique which was originally pioneered for identifying neurodegeneration in diabetic peripheral neuropathy (Petropoulos et al., 2013c, Petropoulos et al., 2014, Petropoulos et al., 2013a, Malik et al., 2003, Ahmed et al., 2012) and subsequently in a range of other peripheral neuropathies (Petropoulos et al., 2019). CCM has recently also been used to identify neuronal injury in a number of central neurodegenerative disorders, including MCI and dementia (Ponirakis et al., 2019a), Parkinson's disease (Misra et

al., 2017), amyotrophic lateral sclerosis (Ferrari et al., 2014) and multiple sclerosis (Petropoulos et al., 2017, Bitirgen et al., 2017b, Mikolajczak et al., 2016). CCM generates *in vivo* images of the sub-basal nerve plexus and image analysis of corneal nerves is performed using validated image analysis software (Dabbah et al., 2011) to reduce inter- and intra-rater variability and quantify corneal nerve morphology (Vagenas et al., 2012, Petropoulos et al., 2013c, Kalteniece et al., 2017).

The objective of this study was to compare the diagnostic accuracy of CCM with MTA rating for MCI and dementia, including AD, VaD and mixed AD.

10.3 Methods

Patients with MCI, dementia, including AD, VaD and mixed AD and no cognitive impairment (NCI) were recruited from the Geriatric and Memory clinic in Rumailah Hospital, Doha, Qatar between 18/09/16 and 31/07/19. Patients with severe anxiety, severe depression, Parkinson's disease, frontotemporal dementia and Lewy body dementia, hypomania, and severe dementia who were unable to cooperate were excluded. Additionally, patients with other potential causes of peripheral neuropathy including vitamin B_{12} deficiency, hypothyroidism, HIV infection and hepatitis C were excluded. Diabetes was not excluded because there is a high prevalence of diabetes in patients aged ≥50 years in Qatar (Bener et al., 2009). Patients with dry eyes, corneal dystrophies, ocular trauma or surgery in the preceding 6 months were excluded. This study was approved by the Institutional Review Board of Weill Cornell Medicine in Qatar and Hamad Medical Corporation and all participants gave informed consent to take part in the study. The research adhered to the tenets of the declaration of Helsinki.

10.3.1 Demographic and metabolic measures

Age, gender, ethnicity, blood pressure, weight, body mass index (BMI), HbA1c, cholesterol, triglycerides, thyroid stimulating hormone (TSH), free thyroxine (FT4) and vitamin B_{12} were recorded.

10.3.2 Cognitive screening

Cognitive screening was performed using the Montreal Cognitive Assessment (MoCA) test. The MoCA assesses seven cognitive domains including visuospatial/executive, naming, memory, attention, language, abstraction and delayed recall giving a total score of 30. A score of $\leq$ 26 indicates cognitive impairment. A point was added for individuals who had formal education ≤6th grade. Cognitive symptom duration was estimated from the clinical history obtained from relatives and participants.

10.3.3 Diagnosis

The diagnosis of MCI and dementia, including AD, VaD and mixed AD were based on the ICD-10 criteria (Organization, 1992). The diagnosis was made according to consensus decision by geriatricians, geriatric psychiatrists and neurologists to exclude reversible, complex and young-onset dementia. The diagnoses of MCI and dementia were based on a patient history and examination, which include (1) presenting complaint and history of illness; (2) comprehensive history of each of the cognitive domains using MoCA; (3) psychiatric history for ruling out depression, mood disorders, and psychosis; (4) medical history including episodes of delirium and other medical comorbidities; (5) medication history; (6) functional history of basic daily living activities. A comprehensive organic work-up including blood tests and brain imaging was undertaken to exclude other potentially reversible causes of cognitive decline such as tumors, subdural hematoma or normal pressure hydrocephalus. The diagnosis of mixed AD was based on the presence of AD and significant vascular changes. Subjects with typical features of AD and no significant decline in functioning were classified as AD. Neuroradiologists blinded to the diagnosis and clinical data assessed for volume loss of hippocampi, entorhinal cortex, and amygdala on MRI, based on the criteria of Dubois et al (Dubois et al., 2009). The diagnosis of probable or possible VaD was based on the NINDS-AIREN criteria (Roman et al., 1993), which include multiple large vessel infarcts or a single strategically placed infarct in the angular gyrus, thalamus, basal forebrain, or posterior (PCA) or anterior cerebral artery (ACA) territories, and multiple basal ganglia and white matter lacunes, extensive periventricular white matter lesions, or combinations thereof.

10.3.4 MRI brain procedures

MRI was performed on a superconductive magnet operated at 3T (Skyra, Siemens). A T1-weighted 3D magnetisation prepared rapid acquisition gradient echo sequence (MPRAGE) was obtained in the sagittal plane with a 1 mm slice thickness, repetition time of 1900 ms, echo time of 2.67 ms and 2.46 ms, inversion time of 1100 ms and 900 ms, flip angle of 9 degree and 15 degree, and FOV= 240 x 100. Coronal and axial reformatted MPRAGE images were made from the sagittal 3D sequence.

10.3.5 Medial temporal lobe atrophy visual rating

A board certified neuroradiologist blinded to diagnosis and clinical data assessed MRI images. T1-coronal images at the level of the midbrain were used to score for right and left medial temporal lobe atrophy (MTA). The right and left hippocampi, entorhinal cortices, perirhinal cortices were separately rated according to the five-point scale developed and validated by Duara et al, and a combined visual MTA score for each hemisphere was calculated averaging the three measurements (Duara et al., 2008). The coronal reformatted MRI slice at the level of the mammillary bodies seen in the sagittal plane was used to define the outline of the medial temporal lobe. The outline of the entorhinal cortex in this slice was defined by the anterior parahippocampal gyrus and adjacent white matter (seen medial to the collateral sulcus and inferior to the hippocampus). The outline of the perirhinal cortex was defined by the fusiform gyrus and adjacent white matter (seen lateral to the collateral sulcus and medial to the occipitotemporal sulcus) (Figure 10.1).

10.3.6 Corneal confocal microscopy

CCM analysis was performed with the Heidelberg Retinal Tomograph III Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany). The cornea was locally anesthetized by instilling 1 drop of 0.4% benoxinate hydrochloride (Chauvin Pharmaceuticals, Chefaro, UK) and Viscotears (Carbomer 980, 0.2%, Novartis, UK) was used as the coupling agent between the cornea and the TomoCap as well as between the TomoCap and the objective lens. Subjects were instructed to fixate on a target with the eye not being examined. Several scans of the sub-basal nerve plexus in the central cornea were captured per eye for ~2 minutes. The field of view of each image is 400X400 µm. At a separate time, three high clarity images per eye were selected by one researcher blind to the patient

diagnosis using established criteria based on depth, focus position and contrast (Kalteniece et al., 2017). Corneal nerve fiber density (CNFD) (fibers/mm^2), branch density (CNBD) (branches/mm^2) and fiber length (CNFL) (total fiber length mm/mm^2) were quantified using CCMetrics, a validated image analysis software (Dabbah et al., 2011).

10.3.7 Statistical analysis

This is an exploratory study as the diagnostic accuracy of CCM for MCI and dementia has not been compared with MTA visual rating before.

Patients' demographics and clinical characteristics were summarized using means and standard deviations for numeric variables and frequency distribution for categorical variables. Variables were compared between the NCI, MCI and dementia group using one-way analysis of variance (ANOVA) with Bonferroni's post hoc test for pairwise comparisons and Chi-square test, respectively.

The neuroradiologist scored for MTA in 30 subjects with NCI (n=10), MCI (n=10), and dementia (n=10), blind to the identity and diagnosis of the subjects. To assess intra-rater reliability, the neuroradiologist repeated ratings in all 30 subjects after an interval of approximately four weeks. Intra-rater reliability was assessed using kappa statistics.

Receiver operating characteristic (ROC) curve analysis was used to determine the ability of CNFD, CNBD, CNFL, MTA-R, and MTA-L to distinguish between patients with MCI and dementia from NCI. The area under the ROC curve (AUC) and a cut-off point with the maximal sensitivity and specificity were calculated.

All analyses were performed using IBM-SPSS (version 23; SPSS Inc, Armonk NY). Dot plots were generated using GraphPad Prism, version 6.05. A two-tailed P value of ≤0.05 was considered significant.

10.4 Results

We enrolled 207 people and excluded 1 patient with severe depression, 1 patient with hypomania and 23 people who did not complete all assessments to leave a sample size of 182.

10.4.1 Demographic and clinical characteristics

182 subjects with NCI (n=36), MCI (n=80) and dementia (n=66) were studied. The demographic and clinical characteristics of these three groups are summarized (Table 10.1). The study cohort comprised of 111 (61.0%) males and 71 (39.0%) females. There were 63 (34.6%) Qatari Arabs, 62 (34.1%) other Arabs, 37 (20.3%) South Asians, and 20 (11.0%) other ethnicities. The prevalence of Type 2 diabetes was 110 (60.4%) and was comparable between subjects with NCI (n=22, 61.1%), MCI (n=46, 57.5%) and dementia (n=42, 63.6%), p=0.71. Gender proportion and the mean age, systolic blood pressure (SBP), diastolic blood pressure (DBP), body weight, BMI, HbA1c, cholesterol and triglycerides were comparable between groups. There was a progressive reduction in cognitive function measured by MoCA between NCI (27.4 $\pm$ 4.1), MCI (22.1 $\pm$ 5.5, p<0.0001) and dementia (12.7 $\pm$ 4.1, p<0.0001) group. The mean duration of cognitive impairment was significantly shorter in the MCI group compared to the dementia group (1.5 $\pm$ 1.6 years vs 3.2 $\pm$ 2.8 years, p<0.0001). The dementia group comprised of AD (n=19, 28.8%), VaD (n=13, 19.7%) and mixed AD and vascular lesions (n=34, 51.5%).

Table 10.1. Demographic and clinical characteristics of the study population.

	NCI (*n* = 36)	MCI (*n* = 80)	Dementia (*n* = 66)	*P* value[1]	*P* value[2]	*P* value[3]
Demographics						
Age, years	71.7 ± 6.2	71.6 ± 5.4	73.9 ± 6.9	NS	NS	NS
Female	11 (30.6%)	34 (42.5%)	26 (39.4%)	NS	NS	NS
Systolic BP, mmHg	140.3 ± 17.0	138.6 ± 17.4	138.6 ± 21.4	NS	NS	NS
Diastolic BP, mmHg	73.7 ± 19.8	71.4 ± 8.3	69.1 ± 10.0	NS	NS	NS
Weight, kg	76.4 ± 10.7	80.7 ± 19.2	75.8 ± 13.8	NS	NS	NS
BMI, Kg/m^2	27.6 ± 4.0	30.6 ± 7.2	30.0 ± 4.9	NS	NS	NS
HbA1c, %	6.7 ± 1.3	7.0 ± 1.7	6.6 ± 1.3	NS	NS	NS
Chol. mmol/L	4.3 ± 1.1	4.3 ± 1.0	3.9 ± 1.2	NS	NS	NS
Trig. mmol/L	1.5 ± 0.7	1.5 ± 0.7	1.4 ± 0.7	NS	NS	NS
Cognitive function						
MoCA	27.4 ± 4.1	22.1 ± 5.5	12.7 ± 4.1	<0.0001	<0.0001	<0.0001
Cognitive impairment duration, years	N/A	1.5±1.6	3.2±2.8			<0.0001
Corneal nerve fiber measures						
CNFD, fibers/mm^2	32.0 ± 7.5	24.5 ± 9.6	20.8 ± 9.3	<0.0001	<0.0001	NS
CNBD, branches/mm^2	90.9 ± 46.5	59.3 ± 35.7	53.9 ± 38.7	0.001	<0.0001	NS
CNFL, mm/mm^2	22.9 ± 6.1	17.2 ± 6.5	15.8 ± 7.4	<0.0001	<0.0001	NS
Medial Temporal Atrophy Measures						
Medial temporal atrophy (right & left)	0.7 ± 0.7	0.6 ± 0.6	2.0 ± 1.0	NS	<0.0001	<0.0001
Medial temporal atrophy (right)	0.6 ± 0.8	0.5 ± 0.6	1.9 ± 1.0	NS	<0.0001	<0.0001
Hippocampus (right)	1.1 ± 1.1	1.3 ± 0.9	2.8 ± 0.9	NS	<0.0001	<0.0001
Entorhinal cortex (right)	0.4 ± 0.9	0.2 ± 0.6	1.6 ± 1.2	NS	<0.0001	<0.0001
Perirhinal cortex (right)	0.3 ± 0.6	0.2 ± 0.5	1.4 ± 1.1	NS	<0.0001	<0.0001
Medial temporal atrophy (left)	0.8 ± 0.8	0.6 ± 0.7	2.1 ± 1.1	NS	<0.0001	<0.0001
Hippocampus (left)	1.3 ± 0.9	1.2 ± 1.0	2.8 ± 0.9	NS	<0.0001	<0.0001
Entorhinal cortex (left)	0.7 ± 0.9	0.3 ± 0.7	1.8 ± 1.3	NS	<0.0001	<0.0001
Perirhinal cortex (left)	0.6 ± 0.7	0.3 ± 0.7	1.8 ± 1.3	NS	<0.0001	<0.0001

[1]*NCI versus MCI.*
[2]*NCI versus Dementia*
[3]*MCI versus Dementia*

Characteristics of 182 participants presented as mean ± standard deviation for numeric variables and frequency distribution for NCI, MCI and dementia. Continuous and categorical variables were compared using one-way ANOVA with Bonferroni's post hoc test and Chi-square test, respectively. Abbreviations: MoCA, Montreal cognitive assessment; NCI, no cognitive impairment, MCI, mild cognitive impairment, CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length.

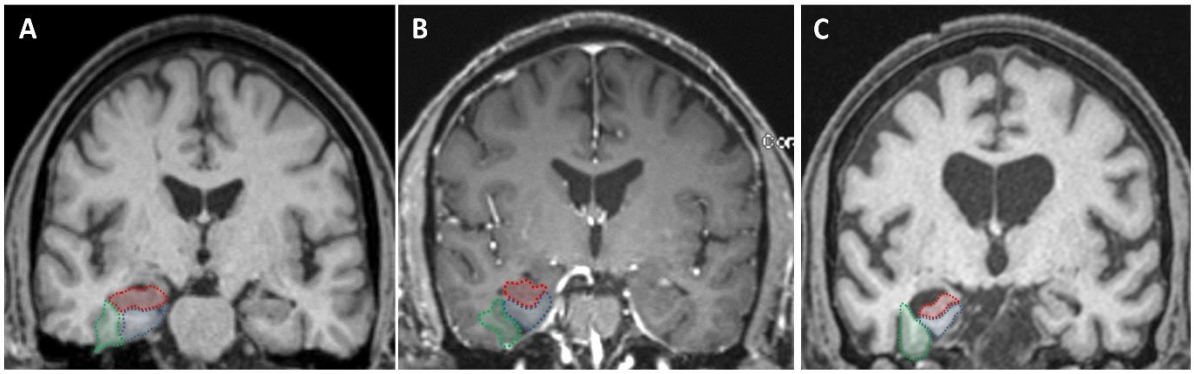
10.4.2 Visual rating of medial temporal lobe atrophy

The inter-rater reliability for MTA rating between two raters was 0.57 and 0.67 for the right and left MTA, respectively. The intra-rater reliability was 1.00 for both the right and left MTA.

The atrophy rating of the right and left hippocampi, entorhinal cortices, perirhinal cortices and medial temporal lobe were comparable between the NCI and MCI group (Figure 10.1 & Table 10.1). The MTA rating of the dementia group was significantly higher compared with

the NCI and MCI group on the right (1.9±1.0 vs 0.5±0.6 and 0.6±0.8, p<0.0001) and left (2.1±1.1 vs 0.6±0.7 and 0.8±0.8, p<0.0001) hemispheres. The average MTA rating in the group with AD (1.9±1.0) and mixed AD with vascular lesions (2.3±1.0) was higher than in the group with VaD (1.5±0.8) but the difference was not significant (P=0.08).

Figure 10.1. Visual rating system for assessing medial temporal atrophy. The three regions of interest are outlined in the right hemisphere in color (hippocampus in magenta; entorhinal cortex in blue; perirhinal cortex in green). Control subject (A) and subject with MCI (B), all showing no atrophy (MTA score=0) in both hemispheres. Subject with dementia (C), all structures have atrophy, (right MTA score=3.3 and left MTA score=2.3).

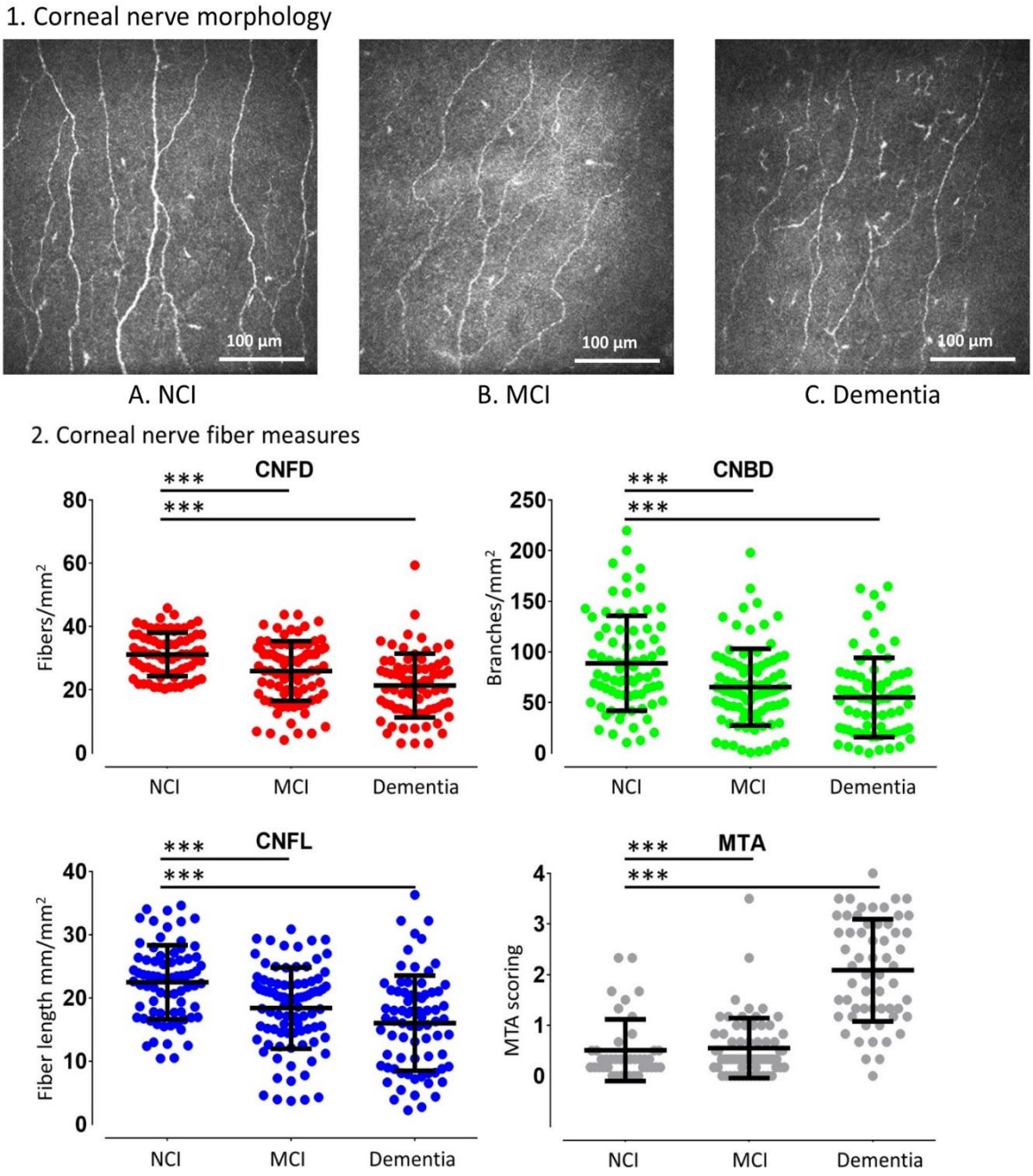


10.4.3 Corneal nerve fiber measures

The corneal nerve fiber measures in subjects with NCI, MCI and dementia are shown in Figure 10.2. Compared to NCI the MCI and dementia group had a significantly lower corneal nerve fiber density (CNFD, fibers/mm^2) (32.0±7.5 vs 24.5±9.6 and 20.8±9.3, p<0.0001), branch density (CNBD, branches/mm^2) (90.9±46.5 vs 59.3±35.7 and 53.9±38.7, p≤0.001) and fiber length (CNFL, mm/mm^2) (22.9±6.1 vs 17.2±6.5 and 15.8±7.4, p<0.0001). CNFD (20.8±10.7 vs 19.8±9.1 vs 21.0±8.8, P=0.93), CNBD (58.1±45.8 vs 51.2±37.2 vs 51.9±36.0, P=0.84) and CNFL (16.4±8.7 vs 15.9±8.4 vs 15.3±6.4, P=0.88) were comparable between subjects with AD, VaD and dementia with mixed AD, respectively.

The difference in corneal nerve fiber measures between subjects with NCI, MCI and dementia remained significant after controlling for T2D (P<0.0001) (Table 10.1). CNFD was significantly different between subjects with and without T2D (P=0.04) but not CNBD (P=0.38) and CNFL (P=0.12).

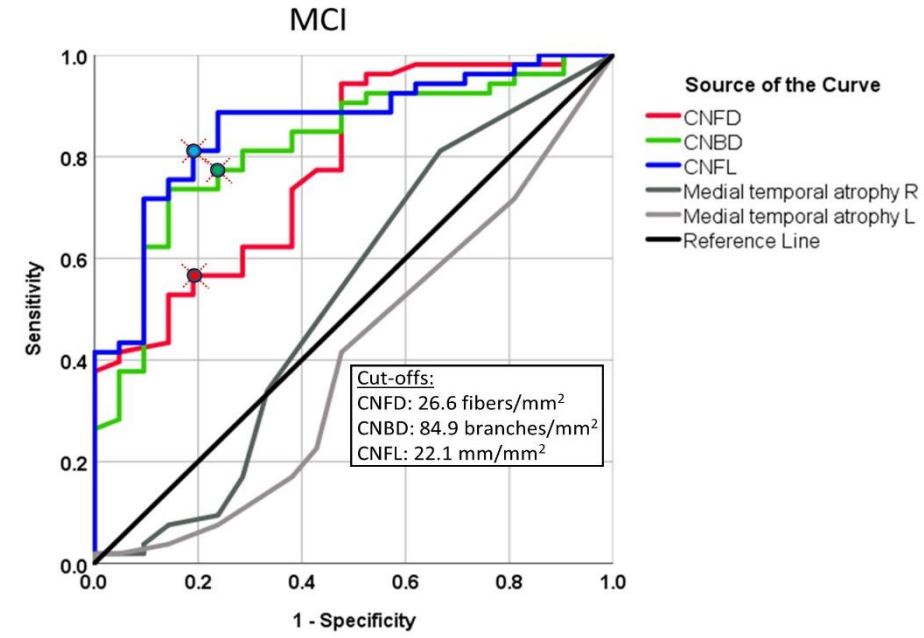
Figure 10.2. Corneal nerve fiber measures, and medial temporal lobe atrophy in subjects with NCI, MCI and dementia. (1) Corneal confocal microscopy (CCM) images of the sub-basal nerve plexus in (A) a 73-year old subject with NCI showing normal corneal nerve fiber morphology; (B) a 69-year old subject with MCI and (C) a 74-year old subject with dementia showing a progressive reduction in corneal nerve fiber density, branch density and length. (2) Dot plots of corneal nerve fiber density (CNFD) (red), branch density (CNBD) (green), fiber length (CNFL) (blue) and MTA scoring (grey) in controls, subjects with MCI and dementia. The line that extends from the middle of the vertical line represents the mean and the lines that extend to the top and bottom are the standard deviation with significant differences between NCI, MCI and dementia group (***P<0.0001).



10.4.4 MTA sensitivity and specificity

The area under the ROC curve (AUC) (95% CI) to distinguish MCI from NCI for MTA-R and MTA-L was not significant 53% (36-69%) and 40% (25-55%), respectively, whilst for dementia it was 86% (76-96%) and 82% (72-92%) ($p<0.0001$), respectively (Figure 10.3 & Table 10.2). The sensitivity and specificity for dementia was 85% and 71% with MTA-R cut-off <0.8 and 79% and 62% with MTA-L cut-off <1.2.

Figure 10.3. The diagnostic accuracy of corneal nerve fiber measures and medial temporal lobe atrophy rating for MCI and dementia. ROC analysis showing the area under the curve for corneal nerve fiber measures and right and left medial temporal lobe atrophy (MTA) rating.



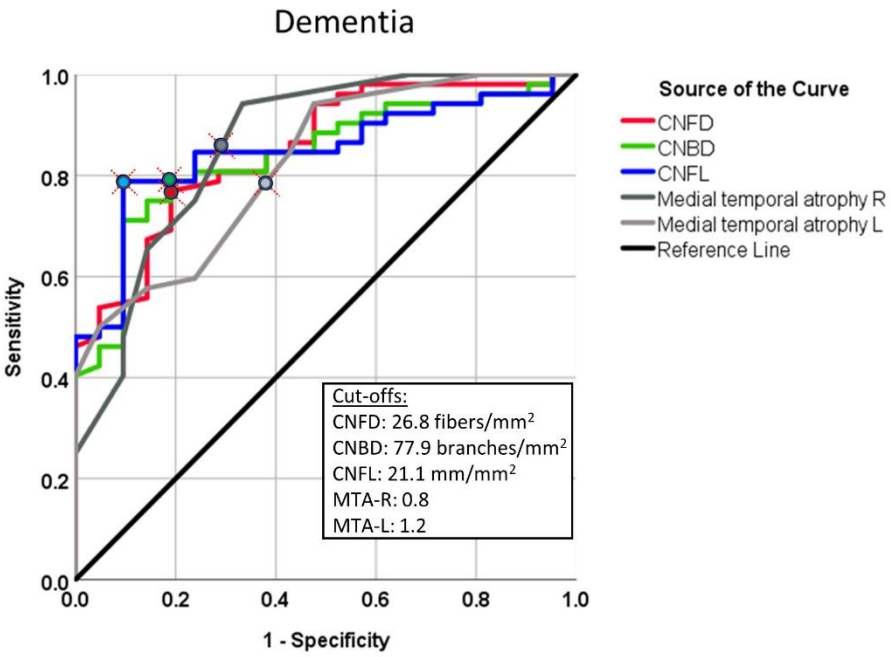


Table 10.2. Receiver operating characteristic (ROC) curve analysis for the diagnostic accuracy of corneal confocal microscopy and medial temporal lobe atrophy rating for MCI and dementia.

	AUC (95% CI)	Cutoff value	Sensitivity	Specificity	*P*-value
NCI vs. MCI					
CNFD, fibers/mm^2	0.78 (0.67-0.90)	26.6	57%	81%	<0.0001
CNBD, branches/mm^2	0.82 (0.72-0.92)	84.9	77%	76%	<0.0001
CNFL, mm/mm^2	0.86 (0.77-0.95)	22.1	81%	81%	<0.0001
MTA-R	0.53 (0.36-0.69)				NS
MTA-L	0.40 (0.25-0.55)				NS
NCI vs. Dementia					
CNFD, fibers/mm^2	0.85 (0.76-0.94)	26.8	77%	81%	<0.0001
CNBD, branches/mm^2	0.84 (0.75-0.93)	77.9	79%	81%	<0.0001
CNFL, mm/mm^2	0.85 (0.76-0.94)	21.1	79%	91%	<0.0001
MTA-R	0.86 (0.76-0.96)	0.8	85%	71%	<0.0001
MTA-L	0.82 (0.72-0.92)	1.2	79%	62%	<0.0001

Abbreviations: no cognitive impairment (NCI), mild cognitive impairment (MCI), corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL), medial temporal atrophy (MTA).

10.4.5 CCM sensitivity and specificity

The area under the ROC curve (95% CI) to distinguish MCI from NCI for CNFD, CNBD, and CNFL was 78% (67-90%), 82% (72-92%), and 86% (77-95%) (p<0.0001), respectively, and for dementia it was 85% (76-94%), 84% (75-93%), and 85% (76-94%) (p<0.0001), respectively (Figure 10.3 and Table 10.2). The sensitivity and specificity for MCI was 57% and 81% with CNFD cut-off <27 fibers/mm^2, 77% and 76% with CNBD cut-off <85 branches/mm^2 and 81% and 81% with a CNFL cut-off <22 mm/mm^2. The sensitivity and specificity for dementia was 77% and 81% with a CNFD cut-off <27 fibers/mm^2, 79% and 81% with a CNBD cut-off <78 branches/mm^2 and 79% and 91% with a CNFL cut-off of <21mm/mm^2.

10.5 Discussion

This study compared the diagnostic accuracy of corneal confocal microscopy (CCM) a non-invasive ophthalmic imaging biomarker of neurodegeneration for mild cognitive impairment (MCI) and dementia (Ponirakis et al., 2019a) with medial temporal lobe atrophy (MTA) rating, an established biomarker for Alzheimer's disease (Albert et al., 2011, McKhann et al., 2011). The diagnostic accuracy of corneal nerve measures of neurodegeneration was high and equivalent to MTA rating for dementia, but it was superior to MTA rating for MCI. MTA rating could not distinguish subjects with MCI from subjects with NCI. Dementia is a

neurodegenerative condition characterized with an insidious onset and a slow progression (Albert et al., 2011). A diagnosis of MCI requires a change in cognition, evidence of impairment in at least one cognitive domain and preserved ability to function independently in daily life (McKhann et al., 2011). However, cognitive assessment tests are influenced by age, educational and cultural background (Albert et al., 2011). A method that allows for greater diagnostic certainty to distinguish normal cognition due to aging from MCI and dementia is required. Biomarkers can support the diagnosis of MCI and dementia by providing direct or indirect evidence of the underlying pathology of the disease and identify subtypes of MCI which do or do not progress to dementia (Albert et al., 2011).

MTA rating as a biomarker of neuronal injury is included in the NIA-AA guidelines to support the diagnosis of AD (Albert et al., 2011, McKhann et al., 2011). Pathological changes occurring in the medial temporal lobe have been demonstrated at autopsy in patients with dementia in the earliest stages of the disease (Barkhof et al., 2007). MTA also occurs in vascular dementia (VaD) but not to the same extent as in AD (Barber et al., 2000, Cho et al., 2009). A gradual accumulation of infarcts or white matter ischemia is associated with hippocampal neuronal loss. In this study, MTA was detected in subjects with AD, VaD and mixed AD and vascular lesions. MTA visual rating was developed for use in clinical practice as it is easy to learn and can be quickly scored to support the diagnosis of AD (van de Pol and Scheltens, 2014). However, there are conflicting data about the diagnostic accuracy of MTA visual rating for AD. Duara et al. (Duara et al., 2008) reported that MTA can discriminate probable AD from no cognitive impairment with a good sensitivity (85%) and specificity (82%), above the 80% threshold (Thies et al., 1999). Heo et al. (Heo et al., 2013) and Cavedo et al. (Cavedo et al., 2014) also reported that MTA scoring has high diagnostic accuracy for AD. Our findings are in line with the study of Falgas et al. (Falgas et al., 2019) showing that MTA visual rating can distinguish between AD and healthy controls with 94% specificity but 77% sensitivity using ≥1.5 cut-off or 90% sensitivity with 56% specificity using ≥1 cut-off. However, previous studies reporting a high diagnostic accuracy for AD with MTA rating assessed patients with late-onset AD who have more atrophy compared to patients with early-onset AD. Furthermore, Duara et al. (Duara et al., 2008) used different MTA visual rating cut-offs for different age groups, ≥2 for 63-75 years and ≥3 for ≥75 years, whilst our cut-off was independent of age. Falgas et al. (Falgas et al., 2019) also reported that MTA rating cannot distinguish patients with early-onset

AD and subjects with MCI. The AUC/sensitivity/specificity were 63%/30%/93% for non-amnesic and 67%/34%/93% for amnesic early-onset AD. In this study, the left and right MTA scores could not distinguish subjects with NCI from MCI.

Corneal nerve morphology has been evaluated using CCM in a number of central neurodegenerative disorders, including MCI and dementia (Ponirakis et al., 2019a), Parkinson's disease (Misra et al., 2017), amyotrophic lateral sclerosis (Ferrari et al., 2014) and multiple sclerosis (Petropoulos et al., 2017, Bitirgen et al., 2017b, Mikolajczak et al., 2016). Previously, we have reported corneal nerve loss associated with cognitive decline and functional independence and reasonable diagnostic accuracy in a smaller cohort of subjects with MCI and dementia (Ponirakis et al., 2019a). In the present study with a greater number of participants we show improved diagnostic accuracy with an AUC (86% vs 73%), sensitivity (81% vs 70%) and specificity (81% vs 75%) for MCI, superior to MTA rating and a comparable AUC (85% vs 86%) and sensitivity (79% vs 85%) but improved specificity (91% vs 75%) for dementia. This study also shows that the severity of corneal nerve loss was comparable between AD, VaD and dementia with mixed AD and vascular lesions.

It is important to account for other causes of corneal nerve fiber loss such as impaired glucose tolerance (Asghar et al., 2014) and diabetes (Azmi et al., 2015). Whilst a large body of data shows that diabetes has a major influence on corneal nerve pathology (Petropoulos et al., 2013c, Petropoulos et al., 2014, Petropoulos et al., 2013a, Malik et al., 2003, Ahmed et al., 2012), diabetes was not excluded from the study as diabetes is more prevalent in people with cognitive impairment and has a high prevalence in patients aged ≥50 years in Qatar (Bener et al., 2009). Indeed, our analysis shows that the difference in corneal nerve fiber measures between subjects with NCI, MCI and dementia remained significant after controlling for diabetes.

Epidemiological studies also show that individuals with T2D have an increased risk of dementia (Zhang et al., 2017a, Gudala et al., 2013). The relative risk for AD and VaD for people with diabetes compared to people without diabetes is 1.53 (95% CI 1.42-1.63) (Zhang et al., 2017a) and 2.27 (95% CI 1.94-2.66) (Gudala et al., 2013), respectively. The increased risk of dementia in patients with T2D is attributed to non-AD mechanisms of neurodegeneration. Diabetes is not associated with excess Aß plaques and neurofibrillary tangles of

hyperphosphorylated tau protein in the brain (Abner et al., 2016, Dos Santos Matioli et al., 2017). However, patients with T2D have a 1.57-times increased odds of an infarct, and 1.71-times increased odds of lacunes in the brain (Abner et al., 2016). Infarcts and lacunes double the risk of dementia occurring within 5 years (Vermeer et al., 2003) and could further decrease cognitive reserve in patients who have accumulating plaques and tangles (Snowdon et al., 1997).

The diagnostic accuracy of MTA visual rating and CCM for MCI should be interpreted with caution because diagnosis of MCI was based on clinical evaluation and cognitive examination using the ICD-10 criteria (International Advisory Group for the Revision of and Behavioural, 2011). This is a significant limitation when comparing the diagnostic accuracy of these two techniques for MCI without biological confirmation of the disease including cerebrospinal fluid (CSF) concentrations of amyloid beta (Aβ) 42, Aβ40, tau/phosphorylated tau (Mattsson et al., 2009, Hansson et al., 2006) or Aβ deposition using positron emission tomography (PET) (Forsberg et al., 2008, Grimmer et al., 2013). This could have led to higher rate of misdiagnosis of MCI. The overlap of corneal nerve measures between MCI and dementia may be attributed to the absence or presence, severity of neurodegeneration and stage of the disease. The optimal role of biomarkers for AD should be to identify the disease in its prodromal stages (Jack et al., 2018). All three corneal nerve measures are reduced in both MCI and dementia and future larger studies may inform us as to which measure is optimal. A longitudinal study is currently underway to compare the prognostic ability of CCM and quantitative brain atrophy on progression of participants with MCI to dementia. CCM may lack specificity for dementia as it occurs in a range of peripheral and central neurodegenerative diseases, therefore future studies should attempt to define specific patterns of corneal nerve fiber alteration in MCI and dementia and assess its utility alongside more specific biomarkers such as Aβ and tau.

In conclusion, this study shows that CCM has high diagnostic accuracy for MCI and dementia, whereas MTA rating has high diagnostic accuracy for dementia but cannot distinguish subjects with NCI from those with MCI. This suggests that CCM is a promising ophthalmic imaging biomarker of neurodegeneration that could be utilized to screen, diagnose and follow up people with MCI and dementia.

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Chapter 11: Conclusions

The prevalence of diabetes in Qatar is almost two-fold higher than the global average of 8.3% and is associated with an increasing prevalence of the long-term complications (IDF Middle East and North Africa Region, 2020, , International Diabetes Federation, 2019,). A common complication of diabetes is diabetic peripheral neuropathy (DPN) a progressive neurodegenerative disorder affecting ~50% of people with diabetes. The clinical diagnosis of DPN is challenging due to the insidious onset of disease and gradual decline of peripheral nerve function (Malik, 2020). It imposes a significant health and economic burden to both the patient and health care providers (Raghav et al., 2018). DPN leads to painful DPN (pDPN) (Ponirakis et al., 2019b), erectile dysfunction (Kouidrat et al., 2017) and diabetic foot ulceration (DFU) (Raghav et al., 2018) in patients with diabetes. Painful DPN has a significant impact on the patient's quality of life (Van Acker et al., 2009, Bohlega et al., 2010) as it is accompanied by depression, anxiety and sleep disturbance (Bohlega et al., 2010).

The prevalence of DPN, pDPN and those at high risk of DFU have not been systematically studied in Qatar. Using a large cohort of randomly selected patients with T2D (n=1,095) attending the two National Diabetes Centers in Qatar, **Chapter 3** shows that the prevalence of DPN was 23%, of whom one-third were at high risk of DFU, and 6% had diabetic foot ulcers. However, 82% of patients with DPN had not been previously diagnosed. **Chapter 4** shows that 1 in 3 patients with T2D had pDPN, but ~80% of patients had not been diagnosed or treated for this condition. **Chapter 5** identified a lower prevalence of DPN and pDPN in primary health care (PHC) compared to secondary health care (SHC), which may be attributed to better overall risk factor control in PHC and referral bias due to patients who are poorly managed with complications being referred to SHC. Alarmingly, ~80% of patients with DPN in PHC were also undiagnosed, highlighting the need for implementing annual DPN screening. PHC had a much lower prevalence of patients with undiagnosed pDPN compared to SHC, which may reflect a more systematic approach to identify neuropathic symptoms as part of a general screen for complications as opposed to SHC where there is no formal screening unless the physician refers for further assessment. Predictors of DPN are age, duration of diabetes, poor

glycemic control, hyperlipidemia and hypertension, whereas for pDPN they are the presence of DPN, obesity, physical activity and smoking. This argues for annual screening and identification of patients with DPN for more aggressive treatment of the identified modifiable risk factors.

Clinical and experimental studies suggest that hypertension is an independent risk factor for DPN in patients with T1D (Tesfaye et al., 2005, Forrest et al., 1997, Cavusoglu et al., 2015, Elliott et al., 2009, Sanada et al., 2015, Gregory et al., 2012) and T2D (Cardoso et al., 2015, De Visser et al., 2014, Kesavamoorthy et al., 2015, Yang et al., 2015). ACE inhibitors have been shown to improve NCS but there are conflicting data about their effect on neuropathic symptoms and other neuropathy measures (Malik et al., 1998, Ruggenenti et al., 2011, Reja et al., 1995). **Chapter 6** assessed the impact of hypertension on both large and small fiber measures in subjects with and without T1D. It shows that hypertension contributes to neuropathy in a cohort of patients with T1D but has no impact in subjects without diabetes. The detrimental impact of hypertension on neuropathy is mediated together with high HbA1c, cholesterol, triglycerides, and BMI. These data also suggest that nerve conduction studies (NCS) should be adopted as the primary endpoints in clinical trials assessing the benefits of blood pressure lowering therapy on DPN.

Most international guidelines recommend metformin after lifestyle intervention for T2D patients. This rational is based on its 40-year long-term safety record and the fact that it has shown a 31% reduced incidence of T2D and 17% reduced incidence of metabolic syndrome at 2.8-years (Knowler et al., 2002). Despite conflicting data regarding the effect of metformin therapy on B_{12} deficiency (Chapman et al., 2016), a number of observational and placebo-controlled studies have confirmed that metformin may reduce vitamin B_{12} levels (Chapman et al., 2016). A potential consequence of B_{12} deficiency is that it could directly result in neuropathy or exacerbate DPN. However, there are conflicting reports on the association between metformin induced B_{12} deficiency and neuropathy, with some reports showing an association (Singh et al., 2013, Roy et al., 2016) whilst others have refuted this (Khan et al., 2017, Russo et al., 2016, Ahmed et al., 2016, Ma et al., 2015). **Chapter 7** shows no difference in B_{12} levels or the severity of DPN or pDPN in metformin compared to non-metformin users. It also shows no difference in DPN or pDPN in those with and without B_{12} deficiency.

There are currently no FDA approved therapies for DPN. There are conflicting data regarding the beneficial effect of GLP-1 receptor agonists on DPN (Kan et al., 2012, Himeno et al., 2011, Jaiswal et al., 2015, Brock et al., 2019). There is evidence showing that pioglitazone might have a neuroprotective effect (Yamagishi et al., 2008). **Chapter 8** is an exploratory sub-study of the Qatar study (Abdul-Ghani et al., 2017), an open-label, randomized controlled trial (clinicaltrials.gov identifier NCT02887625), which showed a rapid and effective reduction in HbA1c after treatment with the combination treatment or basal-bolus insulin in patients with poorly controlled T2D. **Chapter 8** shows that a combination of exenatide once weekly and pioglitazone or basal bolus insulin results in corneal nerve regeneration detecting by CCM, but no change in neuropathic symptoms or sudomotor function. This shows that DPN is amenable to treatment, however, it highlights the importance of selecting appropriate endpoints to show treatment efficacy in clinical trials of DPN.

CCM was originally pioneered for identifying neurodegeneration in DPN (Petropoulos et al., 2013c, Petropoulos et al., 2014, Petropoulos et al., 2013a, Malik et al., 2003, Ahmed et al., 2012) and subsequently in a range of other peripheral neuropathies (Petropoulos et al., 2019) and a large group of healthy people (Tavakoli et al., 2010). It generates *in vivo* images of the sub-basal nerve plexus from which corneal nerve morphology is analysed using validated image analysis software (Dabbah et al., 2011) which reduces inter- and intra-rater variability and enables objective quantification of corneal nerve morphology (Vagenas et al., 2012, Petropoulos et al., 2013c, Kalteniece et al., 2017). CCM has also been used to identify corneal nerve degeneration in a number of central neurodegenerative diseases, including Parkinson's disease (Kass-Iliyya et al., 2015, Podgorny et al., 2016), amyotrophic lateral sclerosis (Ferrari et al., 2014) and multiple sclerosis (Petropoulos et al., 2017, Bitirgen et al., 2017b, Mikolajczak et al., 2016). However, the association between corneal nerve fiber pathology and neurodegeneration in dementia has not been studied. There is an increasing focus on identifying biomarkers for neurodegeneration, which can detect pre-clinical dementia which may be more amenable to disease modifying strategies. Clinical diagnosis of mild cognitive impairment (MCI) or early dementia can be challenging due to the insidious onset of disease and gradual cognitive decline. Biomarkers can support the diagnosis of MCI and dementia by providing direct or indirect evidence of the underlying pathology of the disease. **Chapter 9** a proof-of-concept study shows that CCM identified neurodegeneration, which was associated

with cognitive and functional decline in people with MCI and dementia. Furthermore, the ROC curve analysis shows that CCM might have a good discriminative power to distinguish subjects with MCI or dementia from subjects with no cognitive impairment (NCI). **Chapter 10** compared the diagnostic accuracy of CCM with visual rating of medial temporal lobe atrophy (MTA) using brain MRI to distinguish subjects with MCI or dementia, including Alzheimer's disease, vascular dementia and combined Alzheimer's disease from subjects with NCI. The results show that MTA and CCM have comparable diagnostic ability for dementia, whilst only CCM can distinguish subjects with MCI from those with NCI. This suggests that CCM should be considered as an objective imaging marker of neurodegeneration to support the diagnosis of MCI and dementia.

It is important to account for other causes of corneal nerve fiber loss such as impaired glucose tolerance (Asghar et al., 2014) and diabetes (Azmi et al., 2015). Whilst a large body of data shows that diabetes has a major influence on corneal nerve pathology (Petropoulos et al., 2013c, Petropoulos et al., 2014, Petropoulos et al., 2013a, Malik et al., 2003, Ahmed et al., 2012), diabetes was not excluded from the study for **Chapter 10** as diabetes is highly prevalent in people with cognitive impairment and has a high prevalence in patients aged ≥50 years in Qatar (Bener et al., 2009). Our analysis shows that the difference in corneal nerve fiber measures between subjects with NCI, MCI and dementia remained significant after controlling for diabetes.

Chapter 12: Future work

After the completion of my PhD I plan to find a research and teaching position in academia. I would like to remain with Professor Rayaz Malik's supportive and efficient team to continue the ongoing longitudinal studies in dementia, schizophrenia and diabetes in Qatar. We have established a strong collaboration with the hospitals from which subjects are recruited, Neuroradiology where MRI scans are analysed and Qatar Biomedical Research Institute (QBRI) where transcriptomics, metabolomics and proteomics can be undertaken from the blood samples collected from the studies.

From the **dementia project** I aim to achieve the following objectives:

1. Compare the diagnostic accuracy of CCM with quantitative brain atrophy for MCI and dementia.
2. Determine whether CCM can differentiate subjects with amyloid pathology or AD hypometabolism pattern in subjects with MCI and AD.
3. Assess the impact of vascular lesions and diabetes on CCM measures.
4. Define the change in corneal nerve fiber measures and brain atrophy in subjects with NCI, MCI and dementia over a 2-year period.
5. Compare the prognostic ability of CCM and quantitative brain atrophy on progression to dementia.
6. Determine if a change in corneal nerve measures is associated with a change in cognitive function, disease severity or brain atrophy in subjects with MCI after adjusting for change in metabolic and cardiovascular risk factors over a 2-year period.

From the **Schizophrenia study** I aim to achieve the following objectives:

1. To determine the association of corneal nerve morphology with cognitive function, disease severity and subtype in subjects with schizophrenia.

2. To compare the diagnostic accuracy of CCM with quantitative brain atrophy as a biomarker of neurodegeneration in schizophrenia.

3. To assess the impact of metabolic syndrome on CCM measures in subjects with schizophrenia.

4. To compare the prognostic ability of CCM and quantitative brain atrophy on progression of symptoms in subjects with schizophrenia.

5. To determine if a change in corneal nerve measures is associated with a change in cognitive function, disease severity or quantitative brain atrophy in subjects with schizophrenia after adjusting for a change in metabolic and cardiovascular risk factors.

From the **diabetes study** I aim to achieve the following objectives:

1. Establish a research pathway for subject selection, recruitment and assessment of CCM and DPN in the National Diabetes Center in Hamad General Hospital.
2. Apply for a grant to study the predictive validity of CCM for and the association of change in CCM with 1) progression of DPN symptoms and deficits, 2) diabetic retinopathy and 3) coronary artery disease, peripheral arterial disease and stroke in subjects with diabetes and sub-clinical neuropathy, small fiber neuropathy or those at high risk of DPN based on duration of diabetes, hyperglycemia, hypertension or hyperlipidemia.
3. Undertake further trials to investigate the effect of life-style interventions (i.e. diet and physical activity) or anti-diabetic agents (i.e. Semaglutide and SGLT2 inhibitors) or weight lowering agents (i.e. phentermine-topiramate, bupropion-naltrexone and orlistat) on CCM and DPN measures.

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