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A Comparison of Screening Tools for the accurate diagnosis of Peripheral Neuropathy in Type 2 Diabetes

By

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Abstract

Aim: To investigate and compare different subjective screening modalities recommended in the diabetic foot screening guidelines for detecting peripheral neuropathy in a primary care setting, and compare their results with the objective tool, the NC-Stat® DPN Check®.

Research Design and Methods: A prospective non-experimental quantitative comparative study was conducted in Primary Health Centres. Sixty- three participants (mean age 54.5 years ± 10.5) who met the inclusion criteria and were living with Type 2 diabetes mellitus for at least 10 years were recruited using a convenience sampling method. The subjective tools utilized were the Semmes-Weinstein 10-g monofilament, 128-Hz traditional tuning fork (TTF), neurothesiometer and the O'Brien 128-Hz electronic tuning fork (ETF). These tools were compared with the objective measure NC-Stat® DPN Check® for the detection of peripheral neuropathy. The NC-Stat device was chosen since it has been deemed by research as a reliable tool to detect peripheral neuropathy in its early stages. Each test was carried out bilaterally, therefore a total of 126 limbs were statistically analysed.

Results: A significant difference was reported between all the screening tools when compared in the same group of participants ($P < 0.05$). The descending order of limbs classified as having 'absent' sensation, from highest to lowest percentage is as follows: NC-Stat® DPN Check® (32.5%), ETF constant mode (23.8%), ETF descending mode (23%), TTF (20.6%), neurothesiometer (11.1%) and lastly the 10-g monofilament (4%). Further analysis comparing each subjective tool with the NC-Stat device within their respective categories revealed significant differences between the percentages of limbs with peripheral neuropathy.

Conclusion: The findings have shown that some screening modalities are more sensitive to the diagnosis of DPN than others. This highlights the importance of using multiple screening tools to assess diabetic peripheral neuropathy (DPN) to gain a better understanding of the patient's neurological status. The findings also suggest the inclusion of objective tools such as the NC-Stat tool in diabetic foot screening assessments, as it may enhance the early detection of peripheral neuropathy. Additionally, considering that the subjective measures utilized in this study were all recommended by diabetic foot screening guidelines, the observed variations among these tools suggest a compelling case for change. With the advancements in technology and our evolving understanding of disease progression, it is suggested that these emerging screening tools may be incorporated into revised guidelines to ensure optimal and evidence-based care. Standardizing diagnostic criteria and identifying early biomarkers for nerve degeneration in DPN are crucial for optimal patient care. Further rigorous studies comparing screening tests with a gold standard tool are necessary to determine the most valid non-invasive screening modality utilized in a primary care setting which would reduce the false negative/positive result.

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Lastly, I would like to acknowledge all the individuals who may not be mentioned here but have in some way or another contributed to this research study. Their contributions, whether big or small, are deeply appreciated and have played a significant role in the successful completion of this thesis.

Dedication

I dedicate this dissertation to my cherished loved ones, especially my parents and husband, whose unwavering support and encouragement have been invaluable, driving me to persevere through challenging circumstances and enabling the completion of this study.

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Abbreviations

DPN	Diabetic Peripheral Neuropathy
TTF	Traditional Tuning Fork
ETF	Electronic Tuning Fork
NCS	Nerve Conduction Studies
VPT	Vibration Perception Threshold
IDF	International Diabetes Federation
ADA	American Diabetic Association
BMI	Body Mass Index
DSPN	Distal Symmetric Polyneuropathy
TVT	Timed Vibration Testing
SNCV	Sural Nerve Conduction Velocity
SNAP	Sural Nerve Amplitude Potential
EHES	European Health Examination Survey
LOPS	Loss of Protective Sensation
PAD	Peripheral Arterial Disease
WHO	World Health Organisation

NICE	National Institute for Clinical Excellence
DDG	Deutche Diabetes Gesellschaft
NHMRC	National Health and Medical Research Council
CDA	Canadian Diabetes Association
SIGN	Scottish Intercollegiate Guidelines Network
IWGDF	International Working Group on the Diabetic Foot
NZSSD	New Zealand Society for the Study of Diabetes
SOP	Standard Operating Procedures
DNS	Diabetic Neuropathy Symptom Score
DNE	Diabetic Neuropathy Examination
LDIFLARE	Laser Doppler Imaging Flare
EMG	Electromyography System
TCNS	Toronto Clinical Neuropathy Score

Chapter One

Introduction

1.1 Research Background

The increasing incidence of diabetes mellitus and its complications, both locally and globally, is raising significant concerns (Schembri, 2020). Individuals with chronic uncontrolled diabetes mellitus are more susceptible in developing complications, with one of the most prevalent being diabetic peripheral neuropathy (DPN) in the lower limbs. DPN is characterized by nerve damage to the extremities which is usually irreversible and if left unmanaged can lead to future complications (Ramtahal et al.,2015).

Research has demonstrated that over 50% of the type 2 diabetic population are susceptible to developing DPN with a prevalence rate ranging from 20-30% in those recently diagnosed (Ziegler et al.,2015).

Therefore due to its high incidence, the early detection of DPN is essential to avert subsequent complications and co-morbidities such as ulceration and amputation as well as facilitate an effective treatment plan to prevent further progression and sustain a good quality of life (Yang et al., 2020).

In a local context, diabetes mellitus is a significant contributor to morbidity in Malta. The International Diabetes Federation (IDF) reports that 10.1% of individuals aged 20 to 79 years in Malta are affected by type 2 diabetes mellitus. This therefore highlights the need to develop preventative strategies to manage the morbidity and mortality caused by the many complications of diabetes (Ministry of Energy and Health, 2014); (Schembri, 2020).

Screening guidelines highlight the importance of regular foot examinations and are devised to set recommendations for the diagnosis, prevention and management of complications in diabetes mellitus (Ryden et al., 2013). Various studies have emphasized the significance of implementing a structured diabetes foot screening program, as it has shown to reduce amputation rates by 75%. For this reason, the establishment of evidence-based diabetic foot screening guidelines following standardized protocols is imperative to reduce practice-variability, enhance clinical decision-making and ensure effective screening for optimal patient outcomes (Weck et al., 2013). Moreover, it is important to consider that for effective screening guidelines, the selected modalities should be non-invasive, readily-accessible and cost-effective, while also yielding consistent and accurate results that enable early detection of DPN (Maxim et al., 2014).

However, based on the available literature, it appears that there is a significant variation in the prevalence of DPN across different studies. This variability may be attributed to several factors, such as the lack of consistent diagnostic criteria, variations in the gold standard test utilized, patient awareness, and confounding factors such as the duration of diabetic onset. However, the most crucial factor is the lack of standardized diagnostic criteria as for instance while some studies have utilized the vibration perception threshold (VPT) as gold standard, others have used nerve conduction studies (NCS) (Ramanathan et al.,2021).

Despite the ongoing debate, NCS have been widely regarded by various researchers as the non-invasive gold standard tool for diagnosing DPN in its early stage due to its objectivity and reliability. It produces quantitative measure of conduction through nerve stimulation, however the use of NCS in screening is considered as expensive, time-consuming and limited to specialized practitioners making it not suitable in a clinical setting (Sharma et al.,2015).

Additionally, it is observed that discrepancies exist between guidelines in the methodology of the recommended screening tools due to the absence of universally accepted criteria for their application. These variations can result in the failure to diagnose subclinical cases of peripheral neuropathy (Ramanathan et al.,2021).

The provision of care for individuals with diabetes mellitus remains suboptimal and lacks consistency both within and between countries. The existence of numerous and partially contradictory recommendations and guidelines, as well as the discrepancies between different organizations and countries can lead to confusion among healthcare professionals as well as local-guideline developing bodies. Additionally, the vast number of diabetes foot screening methods proposed can further contribute to ambiguity in clinical care and identification of high-risk patients. Therefore, this emphasizes the need for the development of standardized guidelines for diabetic foot screening to reduce inconsistencies in the diagnosis of DPN (Formosa et al., 2016).

Hence, it is imperative to conduct more rigorous and well-designed research studies comparing recommended screening tests with a gold standard tool to determine and identify the most reliable non-invasive screening modality to be utilized in a primary care setting.

1.2 Justification of the Study

The global prevalence of diabetes mellitus is reaching epidemic levels due to its alarming rise in number (Kharroubi & Darwish, 2015). Given its substantial incidence, it is crucial to implement effective standardized foot screening protocols during routine clinical assessments since patients may be primarily asymptomatic and show initial signs of DPN when advanced complications occur (Pop-Busui, et al., 2017).

Despite extensive knowledge on DPN, there remains a lack of consensus among clinicians and guidelines regarding the optimal assessment method for patients with type 2 diabetes mellitus. The ongoing progress in diagnostic techniques has resulted in variability and consequent ambiguity in clinical practice (Perez-Panero et al., 2019). Furthermore, various studies indicated that the inadequate diagnosis of DPN is often due to a lack of standardization in diagnostic methods. Consequently, management strategies are often implemented only after complications have emerged (Selvarajah et al.,2019).

The Semmes-Weinstein 10-g monofilament is the most widely recommended tool in diabetic foot screening guidelines, where it tests for any sensory impairment by means of light-touch. Its widespread use is due to it being an inexpensive, easily accessible and user-friendly tool (Spruce & Bowling, 2012). However, although commonly utilized, some studies have questioned its sensitivity in diagnosing early stages of DPN (Ang et al.,2018). Therefore, 10-g monofilament was selected as one of the screening tools utilized in this study due to its common use and inclusion in both national and international guidelines for diabetic foot screening.

Some researchers advocate that impaired vibration sensation is usually the first to be affected by diabetic polyneuropathy, which therefore makes vibration perception testing a very important tool in diagnosing DPN (Edmonds, 2020). Indeed, many screening guidelines recommend the use of the 10-g monofilament along with the use of vibration perception testing namely the 128-Hz traditional tuning fork (TTF) or biothesiometer/neurothesiometer (Richard et al.,2012); (Formosa et al.,2016).

Additionally, the American Diabetic Association (ADA) suggests incorporating Timed Vibration Testing (TVT) in diabetic foot screening protocol. The O’Brien 128-Hz Electronic Tuning Fork (ETF) is in agreement with these recommendations for diagnosing DPN (O’Brien & Karem, 2022). Moreover, the IDF included the 128-Hz ETF in its Clinical Practice Recommendation for the Diabetic Foot (Ibrahim, 2017).

Therefore, the vibrating tools namely the 128-Hz TTF, neurothesiometer and 128-Hz ETF were chosen for this study as they are also widely used, user-friendly, readily available and recommended by foot screening guidelines.

Most screening foot guidelines recommend the above valid subjective screening tools due to their availability and ease of use. However, various researchers identified the NCS as the most reliable tool for assessing peripheral neuropathy especially in the early stages although such modalities are not readily available in a clinical setting (Sharma et al.,2015).

The NC-Stat® DPN Check® is a non-invasive objective test which quantifies nerve conduction by measuring the sural nerve conduction velocity and response amplitude. Several research studies have demonstrated the NC-Stat tool exhibits high sensitivity and specificity in identifying the presence of diabetic peripheral neuropathy. These findings highlight the potential of the NC-Stat tool as a promising biomarker for the detection of peripheral neuropathy, supported by strong evidence of its diagnostic accuracy (Lee et al.,2014); (Chatzikomsa et al.,2016). This device may be used to diagnose, stage the severity and monitor peripheral neuropathy before any clinical signs become evident (Carmichael et al.,2021). Furthermore, a study by Shibata, et al. (2019), compared the NCS with the NC-Stat® DPN Check® where a good correlation was exhibited between the two tools. This therefore further supports the efficacy of the NC-Stat® DPN Check® as a diagnostic tool for peripheral neuropathy in individuals living with diabetes mellitus.

Moreover, unlike subjective tools, the NC-Stat tool is not constrained by clinician-dependent interpretation or by limitations in patient cooperation, making it more reliable and objective in assessing peripheral neuropathy (Chatzikosma et al.,2016).

For the purpose of this study, the NC-Stat® DPN Check® was chosen since this non-invasive tool has been proven by various researchers to be valid and reliable as well as able to identify the presence of DPN in asymptomatic patients (Smith & Singleton, 2013).

The results obtained from the NC-Stat tool in this study were compared with the above mentioned subjective clinical modalities commonly used in the primary care setting. The purpose of this study was to investigate whether the available tools used in diabetic foot screening correlate with the findings of the objective sural nerve conduction test for the presence of peripheral neuropathy.

Numerous research studies have aimed to identify the most sensitive non-invasive screening tool for detecting peripheral neuropathy that can be utilized in a primary care setting. However, there is a wide variability among the sensitivity and specificity of these screening tools, primarily because of the lack of standardization in their use and the absence of consensus regarding which tool is considered the gold standard (Ramanathan et al.,2021).

Indeed, several researchers have proposed using a combination of various screening modalities to enhance the detection of peripheral neuropathy. However, there is inconsistency in the screening modalities recommended by different studies (Azzopardi et al.,2018); (Park & Kim, 2019); (Raymond et al.,2020). Additionally, some studies only employed subjective screening tools without comparing them to nerve conduction testing.

Therefore, to date, there is no clinical screening diagnostic tool that has been established as the most effective means of confirming and diagnosing DPN in its early stages apart from NCS (Burgess et al.,2021). Moreover, international screening guidelines are inconsistent in their recommendations on which modality should be used in the early and accurate detection of diabetic peripheral neuropathy (Formosa et al., 2016).

This highlights the importance of more research to determine which tools are the most suitable in the detection of diabetic peripheral neuropathy in its early stages. Furthermore, this research study aims to enhance current knowledge from previous literature which may be significant in shedding light on the current screening guidelines and tools used in the primary care setting.

1.3 Research Statement

There is a lack of agreement with regards to the most suitable and reliable screening tool for the accurate diagnosis of Diabetic Peripheral Neuropathy in a Primary clinical setting.

1.4 Research Question

Are the available screening tools currently recommended in diabetic foot screening guidelines for the detection of peripheral neuropathy consistent with the results obtained from the NC-Stat® DPN Check®?

1.5 Aim & Objectives of the study

1.5.1 Aim

The aim of this study is to evaluate and compare different screening modalities which are currently available and recommended in the diabetic foot screening guidelines for the detection of peripheral neuropathy with the NC-Stat® DPN Check®.

1.5.2 Objective

The objectives of this study include:

- To assess for peripheral neuropathy in patients living with type 2 diabetes mellitus using 4 subjective tools ie Semmes-Weinstein 10-g monofilament, 128-Hz traditional tuning fork, neurothesiometer, 128-Hz Electronic Tuning Fork
- To assess for peripheral neuropathy in patients living with type 2 diabetes mellitus utilizing the objective measure namely the NC-Stat® DPN Check®
- To compare the results obtained from the objective measure with the subjective measures

1.6 Null and Alternative Hypothesis

In a research study, hypothesis testing is a systematic procedure in determining whether the statistical data obtained from a sample population supports the investigator's theory for the entire population. Therefore, the hypothesis test determines the probability of result findings, the possibility of variations or whether the obtained result is too improbable to be considered as a variation chance within a larger scale (Ranganathan & Pramesh, 2019).

There are mainly two types of hypothesis which are the Null Hypothesis and the Alternative hypothesis (Sirisilla, 2022).

1.6.1 Null Hypothesis (H_0)

There is no significant difference in the results obtained from the subjective screening modalities for the diagnosis of peripheral neuropathy ie; 10g Semmes-Weinstein monofilament, 128Hz tuning fork, neurothesiometer, 128Hz Electronic Tuning Fork when compared to the results of the NC-stat tool.

1.6.2 Alternative Hypothesis (H_1)

There is a significant difference in the results obtained from the subjective screening modalities for the diagnosis of peripheral neuropathy ie; 10g Semmes-Weinstein monofilament, 128Hz tuning fork, neurothesiometer, 128Hz Electronic Tuning Fork when compared to the results of the NC-stat tool.

1.7 Dissertation Layout

The dissertation is presented in six chapters:

Chapter One - Introduction

This chapter provides an overview of the current diabetic foot screening guidelines for the diagnosis of peripheral neuropathy. The research background was extensively explored, providing a comprehensive understanding of the existing knowledge and gaps in the field. The justification of the study was presented, outlining the importance of addressing the identified research gaps and the potential impact in clinical practice. Also, the aim, objectives, research question and hypothesis tested were included in this chapter.

Chapter Two - Literature review

An extensive literature review was carried out focusing on the keywords related with this research study regarding the diagnostic tools used to detect diabetic peripheral neuropathy. The review encompassed a wide range of sources, including peer-reviewed journals, books and reputable databases. In this chapter, research relevant to Type 2 diabetes mellitus, overview of diabetic peripheral neuropathy, multiple screening modalities, nerve conduction testing and foot screening guidelines were explored. Through this comprehensive review, key concepts, theories and methodologies utilized in previous studies were critically evaluated, allowing for the identification of strengths, weaknesses and gaps in the existing literature. The aim of this chapter was to establish a strong theoretical foundation and enhance understanding of the research topic.

Chapter Three - Methodology

This section provides a detailed description of the evidence-based methods utilized to carry out data collection as well as the ethical considerations, permissions and recruitment method required prior to commencement of data collection.

Chapter Four - Statistical Analysis

This chapter primarily presented the demographic data collected, followed by statistical analysis conducted by using SPSS software. Tables and charts were utilized to enhance the presentation of the respective findings.

Chapter Five - Discussion

This section encompassed a comprehensive analysis of the research findings, drawing connections with existing literature to provide a thorough and insightful discussion. This chapter also discusses the limitations of this study and any recommendations for future research.

Chapter Six - Conclusion

This final chapter highlights the study findings and warrants for future studies in the subject area.

Chapter Two

Literature Review

2.1 Literature review strategy

A comprehensive literature search should be conducted prior to starting a research study allowing the researcher to critically evaluate any relevant research and their findings. A thorough literature search using online platforms, textbooks and journals will provide background information on the selected area of interest; outline the purpose of the study as well as establishing the need for further research. Furthermore, for a literature review to be effective, the researcher should plan out strategy for the dissertation, conduct thorough research, analyze the findings and extract all the significant research in one review (Young & Layli, 2016).

Prior to commencing the literature search, the research terms used for a study should be identified. The terms chosen were combined using the Boolean operators which help in finding a more focused and fruitful literature required in a research study. Furthermore, the researcher should utilize recent studies and include older articles in a historical context or when new literature is sparse (Mkwebu, 2015).

The following table represents the keywords used in the search engine during the literature search.

Table 1: Salient Terms- Keywords used in this Research Study

Primary Keywords	Secondary Keywords	Tertiary Keywords
• Diabetes Mellitus • Peripheral Diabetic Neuropathy • Diabetic Foot Screening Guidelines • Semmes Weinstein 10-g monofilament • 128-Hz Tuning Fork • Neurothesiometer • 128-Hz Electronic Tuning Fork • NC-Stat® DPN Check®	• Definitions & Classifications • Incidence • Risk factors • Diabetic Foot Complications • Prognosis • Diagnosis	• Limitations • Reliability • Validity • Sensitivity & Specificity • Outcomes

2.1.1 Electronic Search

PubMed, Medline, Journal of the American Podiatric Association, The Diabetic Foot Journal, The Foot, International Journal of Health Sciences as well as other online search engines such as Google, Google Scholar, Medscape and Science Direct were used in the search for any previous literature related to this study. Moreover, HyDi search which is provided by the University of Malta was used to search for online library resources as well full-text journals.

2.1.2 Manual Search

Other supporting literature was also retrieved through Medical Textbooks and printed journals at Faculty of Health Sciences Library. Furthermore, opinions from other medical professionals who are proficient in the chosen field was sought when required.

2.2 Introduction

Lower limb complications such as Diabetic Peripheral Neuropathy (DPN) and/or ischaemia may result in individuals with long term uncontrolled Diabetes Mellitus. However, there are other contributing factors which may lead to causing such complications which include age, smoking, trauma, excessive alcohol/ drug use, infections, Body Mass Index (BMI) amongst others. The presence of these complications consequently affect the patient’s quality of life, cause financial burden, morbidity and mortality (Liu et al.,2019).

DPN is progressive condition and affects an estimate of 50% of the adult diabetic population (Hicks & Selvin, 2019). It is known to be a primary cause of substantial deficits in light-touch sensitivity, vibration perception, lower-limb proprioception and kinesthesia as well as possibly cause severe pain to the lower limbs, foot ulcerations and amputations (Vinik et al., 2014); (Lamparter et al., 2014).

Moreover, patients who developed painful DPN are linked to substantial reductions in general quality of life, heightened levels of anxiety and depression, sleep disturbance and increased gait abnormalities (Akter, 2019).

Although common, DPN is difficult to diagnose as it usually presents with an asymptomatic onset, hence this highlights the importance of a standardized foot screening guidelines to prevent such complications (Dixit & Maiya, 2014).

One of the most common types of DPN is distal symmetric polyneuropathy (DSPN) which generally affects the feet in individuals with longstanding uncontrolled diabetes mellitus. Initially, it starts distally and usually presents bilaterally which emphasizes the significance of conducting a thorough routine screening to identify the presence of DSPN (Kasznicki, 2014)**.**

The diagnosis of peripheral neuropathy can be carried out by conducting clinical screening tests; however researchers have suggested that clinicians should not rule out the presence of neuropathy if the patient is asymptomatic, as up to 50% of DPN initially present with subclinical signs (Pop-Busui et al.,2017).

Therefore, the early diagnosis of neuropathy in patients with diabetes is of extreme importance, as it can prevent many lower limb complications including ulcerations and foot deformities (Burgess et al.,2021). Moreover, DPN can lead to irreversible damage over time making treatment plan for various related complications more difficult (Kluding et al., 2012)**.**

There are various tools available for the diagnosis of DPN, however there is a lack of agreement on which foot screening protocol/guidelines is ideal to implement in clinical practice (Formosa et al.,2016). This ambiguity among clinicians regarding the optimal method of assessing peripheral neuropathy underscores the necessity of standardized guidelines to enhance clinical decision-making and reduce variation in practice as well as in research (Chicharro-Luna et al.,2020).

2.3 Overview Diabetes Mellitus

Diabetes Mellitus is becoming a global concern due to its increase in incidence throughout the years. This condition has been associated with multiple complications causing a higher morbidity and mortality rate as well as affecting various organs in the body leading to irreversible damage or failure (Cheng et al., 2015). Its prevalence has been predicted to increase from 537 million in 2021 to 643 million by 2030 and 783 million by 2045 worldwide (International Diabetes Federation, 2021).

Diabetes Mellitus is a chronic disease responsible for poor control of glucose levels in the body. This most commonly occurs when the body either has deficiencies in insulin production from the pancreas as defined in Type 1 Diabetes or is insulin resistant in both the muscular and hepatic system affecting the beta cell function which is known as Type 2 Diabetes. If left untreated, it can lead to multi-organ and systemic injury which may include cardiac disease, nephropathy, retinopathy, neuropathy and peripheral arterial disease (Casqueiro et al.,2012).

The two main subtypes are Type 1 or Type 2 Diabetes Mellitus, where although both have the potential to cause hyperglycemia, their pathophysiology, presentation and management differ from one another. The majority of individuals with diabetes are Type 2 as it accounts to 90% - 95% of the diabetic population (Russell & Zilliox, 2014).

The below table summarizes the comparisons between the two main subtypes:

Table 2: Comparison between Type 1 & 2 Diabetes Mellitus

Comparison of Type 1 and Type 2 Diabetes mellitus		
Features	**Type 1 Diabetes Mellitus**	**Type 2 Diabetes Mellitus**
Onset	Acute	Insidious
Disease Type	Autoimmune	Metabolic
Age of onset	Typically occurs in Children & adolescents	Typically occurs in Adults
Antibody	Present	Absent
Insulin Sensitivity	Fairly decreased	Severely decreased
Endogenous Insulin	Low or absent	Normal, increased or decreased
Prevalence	Approximately 10%	Approximately 90%
Causes	Strong genetic Predisposition	
Ketoacidosis	Common	Rare
Treatment	Insulin	Diet, Oral hypoglycaemic agents, Insulin
Microvascular Complications (neuropathy, nephropathy & retinopathy)	Yes	Yes
Macrovascular Complications (Cardiovascular & cerebrovascular)	Yes	Yes

(O’Riordan et al.,2009) & (Khan et al.,2013)

The prevalence of diabetes mellitus has been on the rise worldwide due to sedentary lifestyle changes, increase in obesity and aging population. In fact, diabetes is one of the leading chronic conditions worldwide and is expected to increase in numbers in the next few years. In 2021, the global prevalence of diabetes mellitus in individuals aged 20-79 years was estimated to be 10.5% equating to 536.6 million people, with projections indicating a rise to 12.2% (783.2 million) by 2045 (Sun et al., 2022)**.**

Multiple interventional studies have shown that lifestyle modifications and weight loss can effectively prevent the onset of type 2 diabetes mellitus. The Diabetes Prevention Program trial demonstrated that a combination of moderate weight loss, dietary modifications and physical activity resulted in a 58% reduction in the risk of progression from pre-diabetes to type 2 diabetes mellitus (Ackermann et al.,2011).

The increase of diabetes worldwide raises concern as uncontrolled diabetes mellitus has been associated with major health issues as well as causing economic burden to both the individual and the state. Diabetes can also affect a person's functional capacity and quality of life sequentially causing significant morbidity and mortality rates. For this reason, health prevention and screening guidelines have been highlighted in aid to decreasing this global epidemic (Ramtahal et al., 2015).

2.4 Diabetes Mellitus and its prevalence in Malta

Diabetes Mellitus is of great concern in Malta due to its high incidence and upsurge over the years. Its national prevalence of 10.69% is higher than the European average placing Malta in the first quartile in Europe (Cuschieri, 2020). The IDF (2013), projected that by the year 2025, individuals living with Type 2 diabetes will rise from 9.2% to 11.6%. Furthermore, it was estimated an additional 12,000 cases of undiagnosed diabetes mellitus exist in the Maltese population (International Diabetes Federation, 2013).

The main reason of this increase in Malta is due to population growth, ageing and unhealthy lifestyle habit. Over the last few decades, the Maltese population has experienced a notable increase in life expectancy, which has consequently resulted in a higher prevalence of Type 2 diabetes among the elderly population. Also, socioeconomic factors may contribute to the high incidence of diabetes as individuals living in social deprivation in Malta are more likely to adopt unhealthy lifestyle behaviors that increase their risk in developing diabetes later in life (Ministry of Energy and Health, 2014).

Therefore, certain risk factors increase the likelihood of developing type 2 diabetes mellitus. It is crucial to acknowledge that these risk factors can be modifiable, suggesting that individuals can delay or even prevent the onset of type 2 diabetes mellitus. These risk factors are as follows:

- Obesity and unhealthy diet: The majority of adults in developed countries exhibit a continuously increasing trend of overweight and obese individuals, which is attributing to an increase in the diabetic population globally (Bhurosy & Jeewon, 2014). In Malta, compared to other European countries, the male population has the highest rate of overweight and obesity at 69%, while the female population has the third highest rate at 49.1%. Generally, the main cause of obesity is often an unhealthy diet and excessive caloric intake (Ministry of Energy and Health, 2014).
- Sedentary lifestyle: It is well-established that a sedentary lifestyle is a risk factor for type 2 diabetes mellitus and may also lead to overweight and obesity. Lack of physical activity is a risk factor that is worsened by modern trends such as increasing reliance on technology and driving which is particularly evident in developing countries (Park et

al.,2020). Studies conducted by Eurobarometer in 2002 and 2005 have shown that Malta scored poorly in terms of physical activity compared to other European counties, with low scores in average walking duration and daily physical activity duration (Ministry of Energy and Health, 2014).

- Smoking: This is an independent risk factor for type 2 diabetes mellitus, where smokers have a 45% higher incidence of developing type 2 diabetes when compared to non-smokers (Hu, 2011). In Malta, the prevalence of daily smoking among individuals aged 15 and over is 19.2%. Although lower than the European Union with an average of 23.9%, the percentage of smokers in Malta is still quite high (Ministry of Energy and Health, 2014).

It has also been stated that intermarriage amongst the Maltese population and historical background partake in the substantial higher-than-average incidence in Malta (Gatt & Sammut, 2008). Furthermore, the Thrifty Genotype theory explains the history behind the outbreak of diabetes after the Second World War where poverty led to poor nutrition during gestation causing decrease in focal pancreatic cells making the Maltese population more susceptible to diabetes (Poston, 2010)**.**

Furthermore in a study conducted by the European Health Examination Survey (EHES) on Maltese nationals aged 18 year and above has shown that apart from the high incidence of Diabetes mellitus in Malta, there is a notable challenge in effectively managing diabetes. In fact this report found that 57.1% of the sampled population had HbA1c levels exceeding the diagnostic threshold of 6.5%. Among those with diabetes, approximately 62% of females had HbA1c levels exceeding 6.5% which was higher than the 53% observed in males. This suggests that females may have poorer long-term glycemic control compared to males (Directorate for Health and Information and Research, 2012).

The significant prevalence of diabetes coupled with the lack of effectively controlling this condition has a highly economic impact on both the patient and the healthcare institution. This also affects the patient’s quality of life due to the complications arising from diabetes such as ulcerations and amputation in the lower limb. In Malta, it has been reported that the incidence rate of re-ulceration in the diabetic population is that of 32% (Galea et al.,2009). Furthermore, studies have shown that, one out of every four deaths prior to the age of 65 was linked with diabetes in Malta. This highlights the importance of health promotion, clinical screening and early medical interventions (Cachia, 2003).

In 2008, the annual costs associated with diabetes in Malta reached nine million euros. This included expenses for primary care, specialist care and hospital care, with the majority of the costs attributed to prolonged and frequent hospitalizations (Ministry of Energy and Health, 2014). This figure is dated and given the rising prevalence of diabetes in Malta, it is expected to be substantially higher in 2023.

The high incidence of diabetes in the Maltese population highlight the need to establish preventative strategies to manage the morbidity and mortality associated with the various complications of this condition. Therefore, working at the community level is the most effective approach to prevent the onset of diabetes, which underscores the importance of primary care in preventing this chronic condition. Additionally, it is also crucial to reinforce psychosocial support for patients with diabetes and their families as well as fostering great collaboration between the multidisciplinary team and various healthcare sectors (Schembri, 2020).

All Maltese citizens as well as individuals who make social security contributions are entitled to free health care services therefore making most medical services readily available to every person from any socio-economic background. This access to healthcare is crucial for patients living with diabetes as it facilitates improved access to preventive measures which reduces the risk of developing complications (Ministry of Energy and Health, 2014).

Although Malta`s diabetes strategy aims to address and combat the increase in prevalence and complications of diabetes, the incidence of minor amputations in Malta has steadily increased. It has been reported that from an average of 100 minor amputations annually between 2002 and 2006, the total minor amputations between 2015 and 2019 increased to 407 annually, indicating a rate of 229 minor amputations per 100,000 population individuals aged higher than 50 years (Dimech et al.,2021).

The data revealed that a higher number of minor amputations were performed than in any other European country that provided data between 2010 and 2014 to the VASCUNET report (Behrendt et al., 2018). The reasons for this exceptionally high rate of minor amputations are not known, but it could potentially be linked to the high incidence of type 2 diabetes mellitus in Malta coupled with the lack of standardized diabetic foot screening program (Cuschieri, 2018). A reduction of amputations would not only benefit the patients directly but also contribute to indirect benefits for the community and health services (Barshes & Belikin, 2011).

Although lower limb ulcers and gangrene admissions are not solely attributed to diabetes, the majority of them are. In 2010, 192 patients were discharged from Mater Dei hospital due to complications of diabetes related lower limb ulcers/gangrene. The number of discharges increased to 290 in 2011, 223 in 2012 and 322 in 2013, indicating a rising trend of hospital stays due to secondary complications caused by diabetes mellitus (Ministry of Energy and Health, 2014).

This highlights the significance of proper screening for individuals with diabetes to minimize the risk of developing complications. Early detection of diabetes-related complications can enable timely intervention and management, which may prevent or delay any progression. Therefore, regular screening is crucial for individuals with diabetes, especially those with a long-standing history or poor control of their blood sugar levels. Through proper screening, clinicians can identify individuals at risk of developing complications and provide appropriate interventions to prevent their occurrence or reduce their impact (Chicharro-Luna et al.,2020).

2.5 Complications of Diabetes Mellitus in the lower extremity

Diabetes has been associated as a key factor in causing various organ complications throughout the body. Diabetic complications in the lower limbs are common and diverse (Forbes & Cooper, 2013)**.** These complications have been linked with causing morbidity and mortality as well putting a heavy load on the public health services. Lower limb complications are the result from multifaceted interactions between peripheral arterial disease, diabetic neuropathy and structural deformity (Naidoo et al.,2015).

The result of diabetic micro-vascular and macro-vascular damage has increased the probability of patients presenting with infections and ulceration which causes a higher risk of amputation. Research has shown that individuals with diabetes have more than 25 times greater risk of undergoing an amputation than those without diabetes (Pop-Busui et al.,2017).

Furthermore, research has shown that individuals living with diabetes for more than 10 years are more susceptible to develop DPN or/and Peripheral arterial disease in the lower limbs (Pop-Busui et al.,2017), where both of which can cause diabetic foot complications such as ulceration which may lead to amputations. This attributes to the fact that 10% of the diabetic population may develop foot ulceration during their lifetime (Malek et al.,2012), and therefore increasing the risk of morbidity and mortality rates amongst the diabetic population leading to physical, physiological, mental and financial burden for both the patients and public community (Smith-Marsh & Zeller, 2017).

Early and accurate diagnosis of diabetic foot complications will enable clinician to initiate any intervention deemed necessary to minimize deterioration or to prevent any future complications which may affect the patient's quality of life (Boulton et al.,2018).

Routine podiatry screening aims to prevent or decrease the prevalence of foot complications, by making use of foot screening guidelines to evaluate any risk factors that can lead to developing diabetic foot ulcerations (Dorresteijn, 2014). In fact, it has been approximated that 84% of non-traumatic amputations occur following diabetic foot ulceration. Health care professionals work within a multidisciplinary team to implement prevention and management strategies with regards to diabetic foot care. The below illustration (Fig. 1) shows the process which may lead to amputation as well as the link between diabetes mellitus and the risk factors which directly affect the transition between these phases (Barshes, et al., 2013).

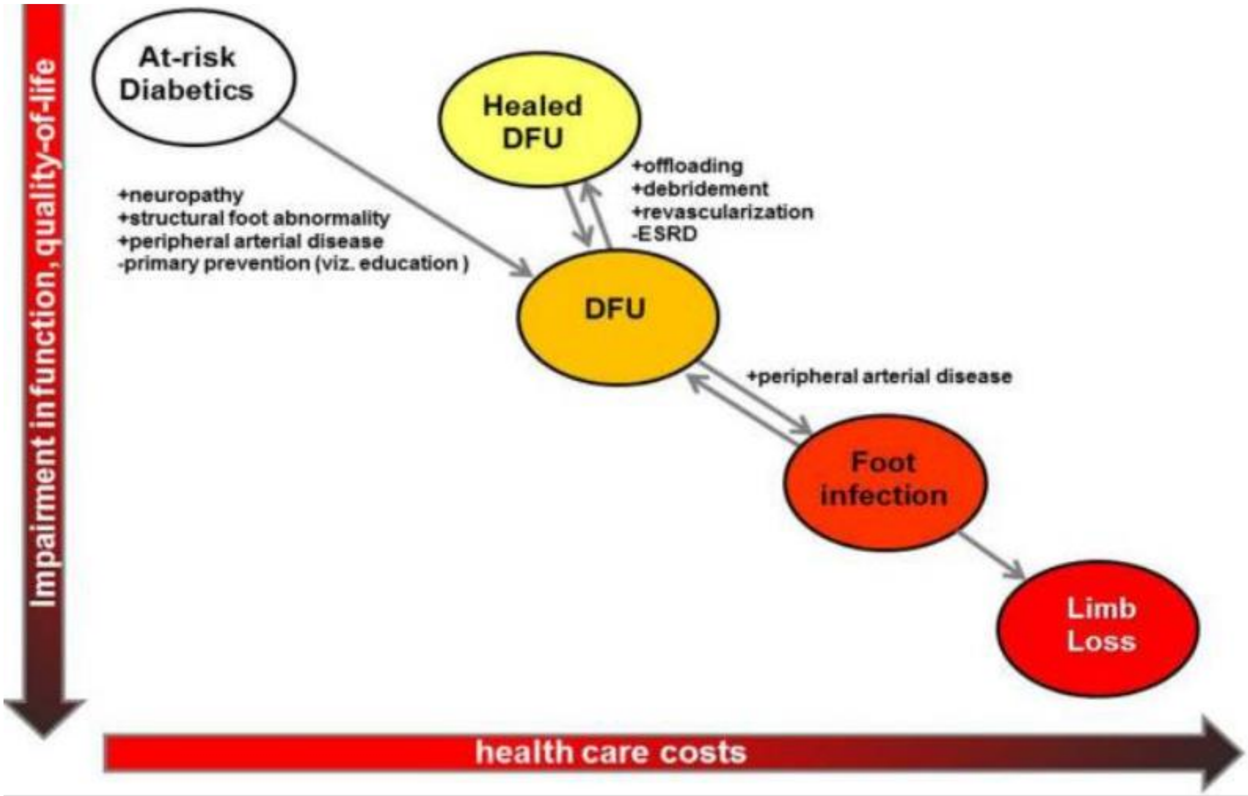


Figure 1- Transition of stages leading to Diabetic amputations

(Barshes, et al., 2013)

2.6 The Peripheral Nervous System

The peripheral nervous system comprises parts of the nervous system which lie outside of the spinal cord and the brain and is responsible to conduct information to and from the central nervous system. These nerve fibers have different functions including motor, sensory and autonomic as well as to support connective tissue and blood supply (Chawla, 2016).

Each neuron consists of three main parts, the cell body, the axon and dendrites as shown in the image below (Fig. 2). Dendrites resemble a tree-like structure, forming finger like projections which can become stimulated and conduct electrochemical charge through the axon to the cell body (Koop & Tadi, 2021).

The unipolar cell bodies of sensory neurons are found within the sensory ganglia, which are enlargements along peripheral nerves of sensory neurons and whose axons form dorsal rootlet that connects to the brain and spinal cord. These can be found along cranial nerves or in the dorsal root of the spinal cord. The receptive field region of neurons is where stimuli can influence the electrical activity of sensory cells and can limit the ability of the sensory nervous system to relay on environmental information. Within the neuron`s receptive field a stimulus can alter the electrical activity of neuron (Papka, 2009); (Koop & Tadi, 2021).

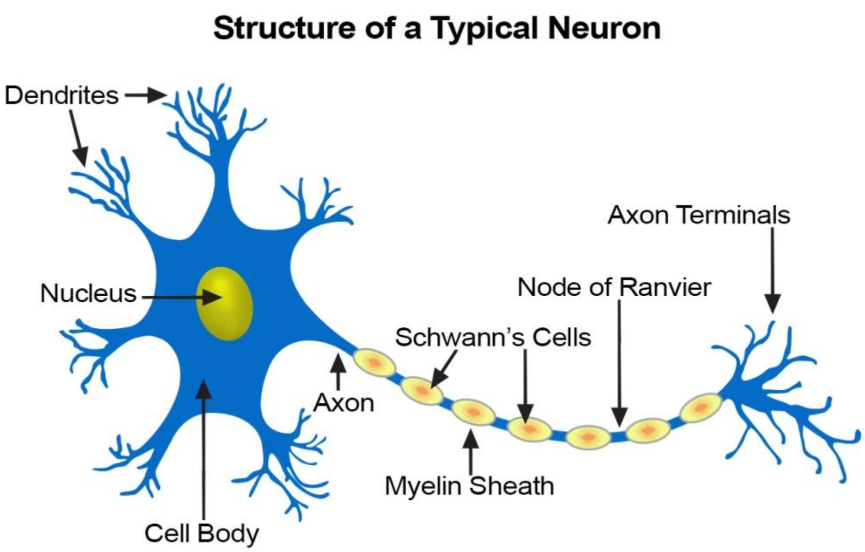


Figure 2- Anatomical Structure of a Neuron

(NIH, 2018)

There are various types of receptors (Fig. 3 & Table 3) in the body where each one of them is responsible for differing stimuli. These include:

A. Thermoreceptors – These receptors are responsible for hot and cold detection, which can be divided into low and high threshold receptors. The warm sensation has been attributed to the function of C fibers, where on the other hand cold sensation is attributed to Aδ fibers (Chu et al.,2022).

B. Mechanoreceptors – Mechanoreceptors can be subdivided into 4 categories the meissner`s corpslues, Pacinian corpuscles, Merkel`s disks and ruffini`s copruscles as described in the image and table below (Fig 3 and Table 3). These receptors are responsible to detect touch sensation, pressure, vibration and cutaneous tension and are considered collectively as low-threshold mechanoreceptors; since even with a weak stimulation of the skin can induce them to produce an action potential. These mechanoreceptors are mostly innervated by relatively large myelinated axons type Aβ; to ensure a rapid central transmission of tactile information (Doll, 2022).

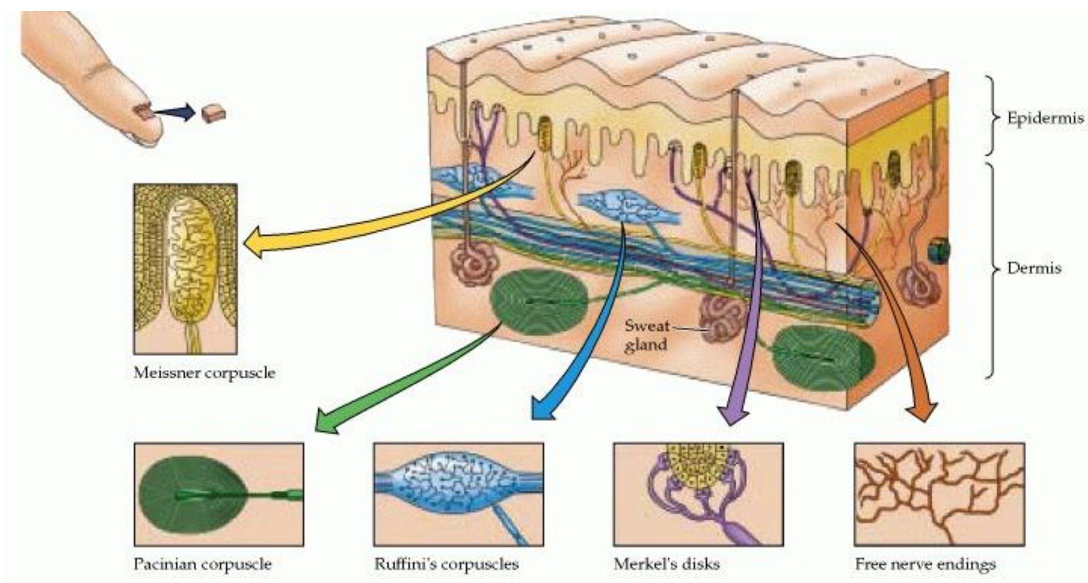


Figure 3- Characteristics in underlying skin

(Purves et al.,2001)

Table 3: Receptor Characteristics

Receptor type	Anatomical characteristics	Associated axons[a] (and diameters)	Axonal conduction velocities	Location	Function	Rate of adaptation	Threshold of activation
Free nerve endings	Minimally specialized nerve endings	C, Aδ	2–20 m/s	All skin	Pain, temperature, crude touch	Slow	High
Meissner's corpuscles	Encapsulated; between dermal papillae	Aβ 6–12 μm		Principally glabrous skin	Touch, pressure (dynamic)	Rapid	Low
Pacinian corpuscles	Encapsulated; onionlike covering	Aβ 6–12 μm		Subcutaneous tissue, interosseous membranes, viscera	Deep pressure, vibration (dynamic)	Rapid	Low
Merkel's disks	Encapsulated; associated with peptide-releasing cells	Aβ		All skin, hair follicles	Touch, pressure (static)	Slow	Low
Ruffini's corpuscles	Encapsulated; oriented along stretch lines	Aβ 6–12 μm		All skin	Stretching of skin	Slow	Low
Muscle spindles	Highly specialized (see Figure 9.5 and Chapter 15)	Ia and II		Muscles	Muscle length	Both slow and rapid	Low
Golgi tendon organs	Highly specialized (see Chapter 15)	Ib		Tendons	Muscle tension	Slow	Low
Joint receptors	Minimally specialized	—		Joints	Joint position	Rapid	Low

(Purves et al.,2001)

C. Nociceptors – These sensory receptors are responsible for detecting signals from damaged tissue; whilst also indirectly respond to chemicals which are released from damaged tissue. These receptors can be found on bones, skin, muscles, joints and viscera and have relatively rapid conduction velocities (Dafny, 2020).

D. Photoreceptors – are receptors which are responsible for detecting light in the retina and convert it into electrical signals which ultimately stimulate a physiological process (Lamkin-Kennard & Popovic, 2019).

E. Chemoreceptors - Chemoreceptors are responsible for obtaining information about the chemical environment and subsequently conveying the information to neurons.

Each receptor (Fig. 4) within a specific field would respond to stimuli by generating electrical impulse along the associated first-order neuron through an action potential. Sensory nerves consist of different type of fibers which depend on their associated receptors. The classification of sensory nerves includes the numerical or Gasser system (Watson & Dyck, 2015).

Proprioceptors which are located in subcutaneous tissue are capable of detecting motion through a stimulus produced by the body and receive innervation from type Ia (A-alpha), Ib (A-alpha) and II (A-beta) sensory fibers. These fibers are myelinated and have a large diameter when compared to other nerve fibers, which contribute to rapid conduction velocity (Beran, 2015)

Conversely, nociceptors and thermoceptors are innervated by type III and IV – C fibers. The C fibers are unmyelinated and smaller in diameter and require a greater threshold of stimulus than A- delta fibers. These fibers are responsible for slower onset of deeper pain, which usually follow an initial insult relay from A-delta which are thinly myelinated and conduct information which is primary related to acute pain, in order to facilitate a withdrawal reflex (Beran, 2015).

As a general rule, the larger diameter of the axon and the myelination of the axon the higher conduction velocity, since large fibers have less resistance facing the ion flow whilst, myelination promotes rapid impulse transmission (Freeman et al.,2016).

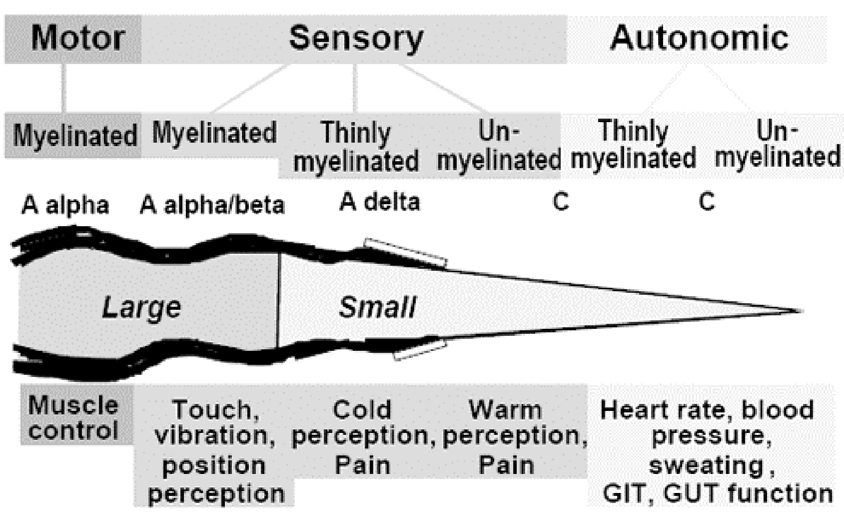


Figure 4- Motor, Sensory and Autonomic Nerve Fibers

(Vinik, et al., 2006)

2.6.1 Supportive structure of Nerve Fibers

The nerve fibers are maintained in a supportive structure which included the mesoneurium, epineurium, endoneurium, perineurium and myelin sheath (Schraut, et al., 2016) as shown in the image below (Fig. 5).

The mesoneurium is the outer loose connective tissue surrounding the peripheral nerve, which suspends the nerve trunk and is continuous with the underlying layer called the epineurium. The epineurium compromises extrinsic blood vessels, whilst further internal plexuses lie in the epineurium, perineurium and endoneurium (Schraut, et al., 2016).

The interfascicular epineurium which consists of longitudinal collagen fibers is an important connective tissue structure as it protects the nerve trunk against mechanical stress which can damage the nerve. The second most inner layer covers individual fascicles of axons and is called the perineurium.

Finally, the endoneurium is the most inner supportive structure of the nerve and it covers directly the individual axons. Furthermore, schwann cells are responsible in insulating individual axons of the peripheral nervous system with myelin except for C fibers (Koop & Tadi, 2021).

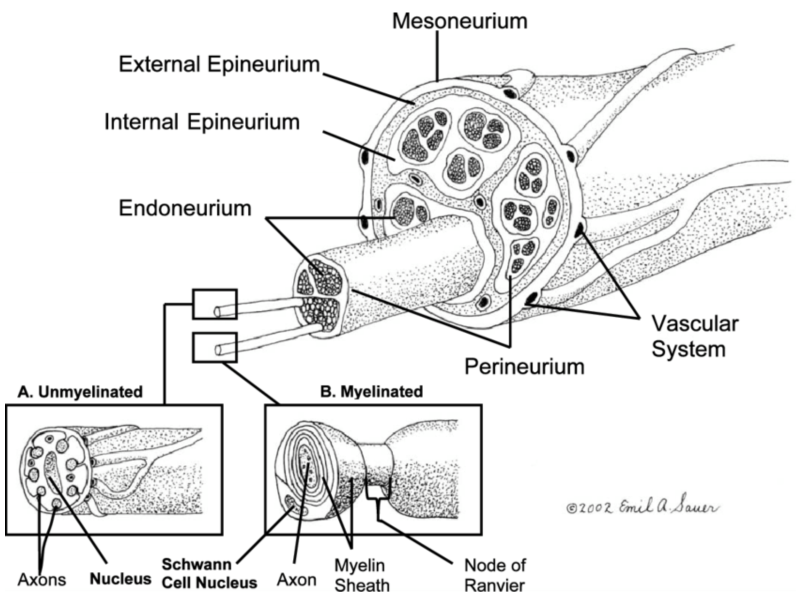


Figure 5- Peripheral Nerve Anatomy

(Landers & Altenburger, 2003)

2.6.2 Sural Nerve Anatomy

The sural nerve is a peripheral nerve which has its origins within the sciatic nerve, and branches from the tibial and common peroneal nerve in the superficial aspect of the distal third of the leg (S1, S2) (Miniato & Nedeff, 2021). The sural nerve passes from the mid-popliteal fossa area along the lateral side of the leg, posteriorly to the lateral malleolus and reaches the 5th toe as the lateral dorsal cuteneuos nerve as shown in the image below (Fig. 6) (Chaudhari et al.,2017).

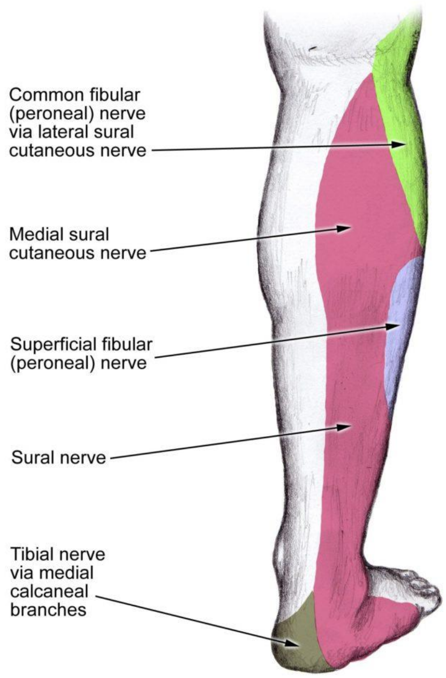


Figure 6- Nerve innervation

(Pivotal Motion, 2019)

The sural nerve has a purely sensory function, and supplies sensation to the skin of the lateral side of the foot and the 5th toe. Since it is one of the distal sensory nerve of the lower limb, it is more likely to be affected early by length dependent peripheral neuropathies such as DPN (Sreenivasan et al.,2016).

Furthermore, the sural nerve can be easily located anatomically, which makes it ideal for nerve conduction testing as it is easily accessible, and less prone to damage caused by local trauma or entrapment when compared to plantar and interdigital nerves, which also pose more technical difficulties to examine. Therefore, the sural nerve is useful in the early detection of peripheral neuropathy, through the use of nerve conduction testing which would help in the possible diagnosis of early subclinical distal peripheral neuropathy (Chatzikosma et al.,2016).

2.7 Diabetic Peripheral Neuropathy

Individuals living with uncontrolled diabetes have a high incidence of developing neuropathy which is a serious diabetic complication. Research has shown that about 45% of individuals with type 2 diabetes have a prevalence of developing neuropathy and in type 1 diabetes individuals the probability of neuropathy is 54%- 59% (Zilliox & Russell, 2011).

Peripheral neuropathy is characterized by damage to the peripheral nervous system, resulting in compromised nerve conduction between the central nervous system to the rest of the body. The manifestation of symptoms may vary from one patient to another and encompass a spectrum from mild to disabling. The severity and type of damage incurred, as well as the specific nerve fibre affected play a crucial role in determining the range of symptoms experienced (Toft, 2021).

This heterogeneous condition affects different areas of the nervous system and has various clinical or subclinical presentations. There are various forms of diabetic neuropathies which affect the lower limb namely Peripheral sensory neuropathy, Proximal neuropathy and Autonomic neuropathy. The table below (Table 4) illustrates the differences between the neuropathies which can all be the result of poor glycaemic control (Toft, 2021).

Table 4: Classification of Neuropathies in the Lower Limb

Classification	Peripheral Sensory Neuropathy	Proximal Neuropathy (aka Diabetic Amyotrophy)	Autonomic Neuropathy
Nerve affected	Sensory Nerves	Motor Nerves	Autonomic Nerves
Nerve function	Sensation such as light touch, temperature, pain and vibration	Controls muscle movement such as ambulation	Maintains homeostasis such as thermoregulation via sweat glands and blood vessels
Symptoms of nerve damage	Burning, Tingling, insensate, inability to feel pain, loss of vibration perception and temperature changes	Muscle weakness such as cramping, contraction of digits, prominence of metatarsal heads and intrinsic muscle atrophy of the feet	Affecting diaphoresis, denervation of peripheral blood vessels resulting in calcification and demineralize bone leading to Charcot foot

(Toft, 2021)

DPN is the most prevalent type of neuropathy worldwide and is commonly associated with the duration of diabetes especially if poorly controlled. It affects around 50% of patients diagnosed with diabetes mellitus. DPN is predominantly sensory and symmetric in nature, typically beginning at the distal aspect of the limbs and gradually going proximally (Iqbal, et al., 2018).

In individuals with type 1 diabetes, the prevalence of DPN is 6% during the onset of the disease, which increases to 30% after 13-14 years of progression (Hicks & Selvin, 2019). The prevalence is slightly higher in individuals with type 2 diabetes, with a presence of 26% in young patients with type 2 diabetes (Sempere-Bigorra et al.,2021).

Furthermore, in patients with type 2 diabetes, DPN could already present at the time of diagnosis and its incidence increases with both the patient's age and diabetic duration. Therefore, according to the literature, it is evident that the incidence of DPN is expected to increase in the upcoming years. As a result, it is essential to promptly detect neuropathy and provide the patient with the most appropriate therapeutic interventions to prevent or delay the development of complications or to manage the symptoms effectively (Galiero, et al., 2023).

2.7.1 Pathophysiology of Peripheral Neuropathy

Peripheral neuropathy is most commonly caused by long standing hyperglycemia. It has been suggested the pathophysiology of peripheral neuropathy is multifactorial although the exact cause is still uncertain. Researchers have provided multiple theories possibly explaining the process of DPN at a molecular level (Buchman, 2010).

The polyol pathway theory stated that individuals with uncontrolled diabetes result in having excess intra-neuronal glucose levels. Following this, an alternative catabolic pathway is initiated to convert glucose to sorbitol with the final conversion to fructose in the aim of balancing these excess levels. The result of these oxidative reactions generates high stress levels which metabolically damages the neurons therefore affecting nerve function (Niimi et al.,2021).

Another theory proposes that the intracellular hyperglycaemia which activates the kinase C β-2 protein is responsible for nerve damage. When a high amount of protein is present, it causes a sequence of physiological effects namely increase in basement membrane matrix protein deposits, activates leucocytes as well as proliferates and contracts the smooth muscle. The mentioned effects result in reducing endoneural circulation leading to nerve damage (Mochly et al.,2012).

The final theory accentuates the involvement of advance glycation end-products (AGEs) as a precursor to the pathophysiological process of neuropathy. High glucose levels initiate a chain of complex transitional process namely non-enzymatic glycosylation of proteins resulting in the formation of AGEs. An increase in the accumulation of AGEs lead to the unintentional deposition in nerves which results in endoneurial thickening of vessels walls inhibiting nerve microcirculation (Singh et al.,2014).

Although, peripheral neuropathy is mainly due to hyperglycaemia and prolonged onset of diabetes mellitus (Ang et al., 2014), there are other potential risk factors which can cause peripheral neuropathy such as:

Age: A study conducted by Brisset & Nicolas (2018), showed that neuropathy is highly prevalent among individuals aged over 65 years, with an increased prevalence with advancing age. The prevalence of polyneuropathies is approximately 7% in the elderly population. However, above 80 years of age, the likelihood of finding no identifiable cause is approximately 40%, which required further explorations. Another study found that peripheral neuropathy affects 10% of the general population, and the incidence increases to 30% in patients aged over 65 years, indicating that the prevalence of developing peripheral neuropathy increases with age (Hicks et al.,2021).

Peripheral arterial disease: Early vascular disease is a common etiology of DPN, which is a condition characterized by reduced or compromised blood flow in the blood vessels.

Peripheral arterial disease can lead to ischaemic tissue damage, which in turn can cause axonal degeneration and results in axonal polyneuropathy (Kim et al.,2014).

Height: In the context of peripheral neuropathy, height is a significant and practical predictor. This is due to the positive correlation between height and the length of nerve fibers, which results in a larger surface area of axons available for toxin exposure and physical damage. Therefore, the risk of peripheral neuropathy is greater in individuals with increased height (Kote et al.,2013).

Obesity: It is an emerging risk factor for neuropathy that is not dependent on hyperglcaemia. This could be attributed to metabolic changes or nerve compression that may predispose them to clinical neuropathy in the future (Callaghan, 2020).

Other conditions: There are conditions which may cause peripheral neuropathy in the lower limb which include alcoholism, drug use, Hepatitis B or C, HIV, Lower back injury/compression, pregnancy, degenerative disorders and many more (Huang et al.,2016); (Brown et al.,2017).

2.7.2 Distal Symmetric Polyneuropathy (DSPN)

The most common chronic complication in individuals living with diabetes is distal symmetric polyneuropathy (DSPN) which accounts to 75% of diabetic neuropathies. DSPN is a symmetrical length-dependent distal sensorimotor polyneuropathy that typically manifest in individuals with long standing uncontrolled diabetes, whilst it can also result from a major trauma, cardiovascular risk covariates or micro vessel damage (Dyck, et al., 2011).

Clinically, DSPN can present with signs and symptoms of nerve damage in the peripheries caused by an abnormality in nerve conduction or may initially be asymptomatic. For this reason, it is important to identify the presence of neuropathy by performing multiple quantitative tests to confirm its diagnosis as well as its severity (Dyck, et al., 2011). Symptoms vary depending on which sensory fiber is being affected therefore dividing it into two subtypes namely small-fiber neuropathy and large-fiber neuropathy (Barrett, et al., 2017).

When damage to the large sensory fiber occurs, individuals experience impaired vibration perception and touch which may occur in both hands and feet. Other sensory symptoms also include numbness, pins and needles, tingling, ataxia and loss of proprioception. It may also contribute in the loss of reflexes and muscle weakness making individuals unable to coordinate complex movements such as affecting gait which increases risk of falls and fractures (Barrett, et al., 2017).

On the other hand, small fiber polyneuropathy affects unmyelinated nerve fibers and when these nerves are effected the ability to feel pain, incur burning sensation or thermal imperceptions may be effected. This can also lead to neuropathic pain which can get worse during the night affecting the individual's sleep patterns, and occur when pain receptors are triggered spontaneously leading to allodynia which is severe pain from even light touch. Therefore, this challenges clinicians in finding ways to ease symptoms caused by small fiber polyneuropathy as it usually greatly affects the individual's quality of life and may even lead to morbidity and mortality (Hobaguimian & Gibbons, 2012).

2.7.3 Management of Diabetic Peripheral Neuropathy

DPN is frequently misdiagnosed and inadequately managed. Currently, there are no approved pathogenic treatments for diabetic neuropathy, aside from optimizing glycaemic control (Kasznicki, 2014).

Peripheral neuropathy can cause multiple deformities in the foot which may result in ulceration or amputation. Neuropathic patients may develop mechanical and structural abnormalities in the lower limbs. These abnormalities affect the person's biomechanics as well as increase areas of high pressures in the foot which can lead to tissue breakdown and ulceration (Formosa et al.,2013).

Change in the forefoot structure secondary to peripheral neuropathy can also occur causing toe contracture such as hammer toes or clawed toes as well as limit mobility in the joints and increase metatarsal prominence. The changes in foot structure increase plantar pressure, prolong repetitive trauma and form callosities making it more prone to develop foot ulceration (Cheuy et al.,2016).

Additionally, peripheral neuropathy can cause loss of sensation making individuals unable to feel pain such as accidentally stepping on a foreign object or trauma to the skin and if left untreated can lead to ulceration with the possibility of an amputation (Gomatos & Rehman, 2022).

Another foot condition secondary to diabetic neuropathy is Charcot foot disease which is a rare, life-threatening progressive destructive arthropathy resulting in severe foot deformity mainly of the metatarsal-tarsal joints. Early detection is vital as this complication is subtle and difficult to diagnose until signs of severe structural deformity transpires. This condition increases the risk of developing ulcerations, osteomyelitis and amputations leading to severe deformities (Armstrong et al.,2017).

Clinical signs indicating Charcot foot usually present unilaterally and include increase in skin temperature and severe oedema. Diagnosis of this condition can be confirmed through radiograph imaging by noting the following features which include fractures, osteolysis fragmentation, ossification, subluxation and joint dislocation and can subsequently result in ulcerations (Trieb, 2016) (Fig. 7).

The prevalence of a diabetic ulcer forming is between 19% to 34% in a lifetime of an individual with diabetes. Furthermore, approximately 20% of individuals with diabetic foot ulcer were required to undergo a minor or major amputation (Armstrong et al.,2017); where a mortality rate of 10% was reported one year following diagnosis of a diabetic foot ulcer (Hoffstad et al.,2015). Moreover, mortality rates increase following diabetic foot amputation, where after 5 years 54% to 79% are deceased after minor amputation whereas 53% to 91.7% die following major amputation (Yammine, 2020).; (Harris & Fand, 2021).

It is approximated that within a diabetic population, 40% - 60% of ulcers are neuropathic in nature and 24 % are solely ischaemic whilst 16% are neuro-ischaemic ulcers (Edmonds et al.,2021).

Although the formation of ulceration can be multifactorial, the most common cause is unperceived trauma. Individuals with loss of sensation in the lower limb are more susceptible to developing foot injuries which may go unnoticed. Ulcerations are a portal of entry for infections which if left untreated may cause osteomyelitis or in severe cases develop cellulitis which can effuse the underlying structures leading to amputation (Boulton, 2013).

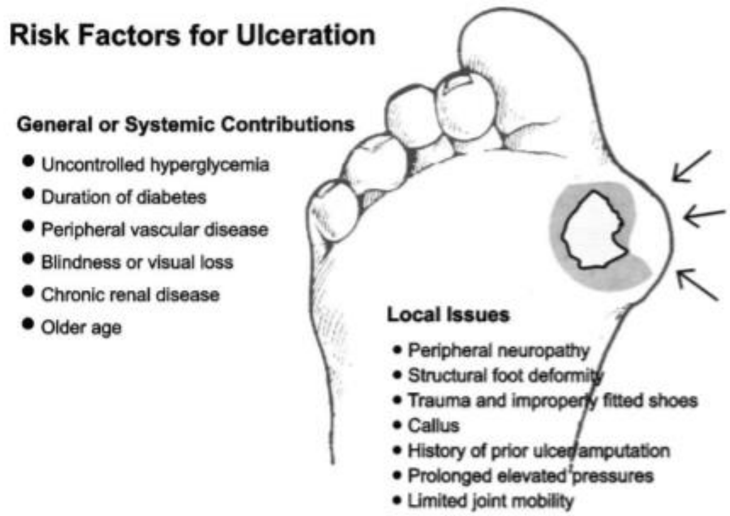


Figure 7- Risk Factors for ulceration

(Yazdanpanah et al., 2015)

Painful neuropathy affects a considerable proportion of the diabetic population, with prevalence ranging between 10-26% depending on the sample population. Painful DPN is notably more frequent in type 2 diabetes compared to type 1. The management of painful DPN presents a challenge due to the complexity of personalizing therapy and determining the optimal initial pharmacotherapy, most effective dosing strategy, considering combination therapy and establishing treatment protocols for patients who do not respond well to analgesics (Iqbal et al.,2018).

Neurological deficits and painful neuropathy are both associated with a decreased quality of life and increased risk of mortality (Fig. 8) (Kasznicki, 2014).

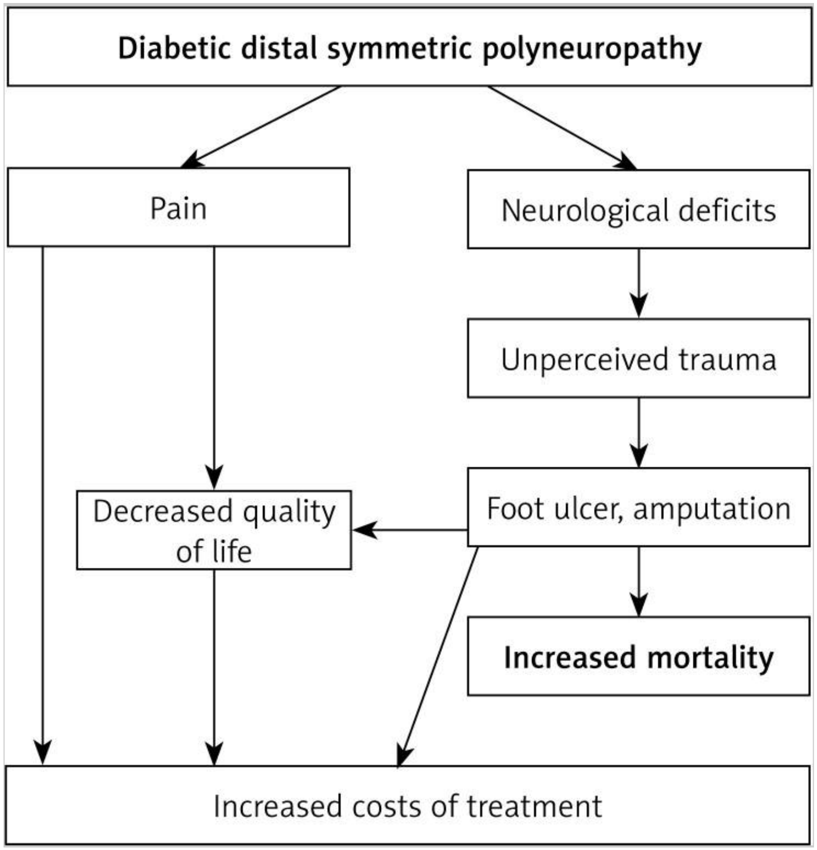


Figure 8- Burden of DSPN

(Kasznicki, 2014)

Interventions are person-dependent where the clinician formulates a treatment plan to prevent complications from arising. When dealing with individuals suffering from peripheral neuropathy, it is essential that clinicians focus on foot care advice, general health education whilst performing routine screening as well as formulating a treatment plan tailored to the patient's need. Furthermore, research has shown that lack of awareness has caused unnecessary morbidity therefore advice on lifestyle changes and glycaemic control is important to decrease risks associated with neuropathy. Other treatment plans may include managing excessive plantar pressure, therapeutic footwear, ulcer management, pain medication and advice on frequent foot inspection to avoid deterioration of tissue breakdown (McDermott et al.,2022).

2.8 Foot Screening Guidelines for Peripheral Diabetic Neuropathy

The goal of optimal diabetic care is to prevent and manage diabetic foot complications, which can be achieved through early detection and intervention guided by clinical guidelines (McDermott et al.,2022).

Although diabetes mellitus can cause serious irreversible complications, it can easily be managed with good glycaemic control and implementation of proper management. It is essential that individuals living with diabetes are educated on the nature, treatment, regular screening and complications which may arise if condition is not controlled. Therefore, diabetic care education and knowledge has shown to be beneficial in decreasing complication and minimizing risk of morbidity and mortality (Nazar et al., 2015).

In the past few years, a notable improvement on the significance of diabetic foot care has been observed since there is a better understanding on the causal factors leading to amputation. Foot complications are a major reason of morbidity and a prevalent cause of hospitalization amongst the diabetic population-(Oliver & Mutluoglu, 2022).

Clinical guidelines are a set of recommendations to clinicians for the prevention, diagnosis and management of diabetes which are intended to reach a standardization of care to patients. These guidelines are formulated through evidence-based literature and usually focus on general diabetic care (Aschner et al.,2016).

Studies have shown that early foot screening, proper clinical guidelines and prompt management prevent 45-85% of amputations from occurring. Therefore, to achieve this goal in decreasing amputations, clinicians should formulate a great understanding to the risk factor contributing to these complications (Hinkes, 2014).

Health institutions and researchers have tried to develop a set of guidelines and standards by using evidence-based research with the goal of optimizing prevention, diagnosis and management of diabetes mellitus. The formulation and implementation of proper diabetic foot clinical guidelines is essential as it could decrease amputation rates by 75% (Weck et al.,2013).

These screening guidelines will help the clinician to identify asymptomatic patients who are at high risk in developing complications by making use of the proper screening modalities. This will therefore ensure that individuals with diabetes receive the best quality in care when following such guidelines (International Diabetes Federation, 2015).

Unfortunately, there is no general agreement when it comes to clinical guidelines in diabetes mellitus and each country has set their own guidelines individually (Parker et al.,2019).

In fact, many international guidelines for diabetes foot screening suggest different modalities in determining and diagnosing foot risk. Therefore, this may cause confusion when deciding which recommendations the clinician and the organizations should follow (Formosa et al.,2019).

Therefore, standardized foot screening guidelines for DPN are important for several reasons which include:

- Early detection: Standardized foot screening guidelines can help healthcare professionals to detect peripheral neuropathy early, before it progresses and cause irreversible damage (McDermott et al.,2022).
- Consistency: Standardized guidelines should ensure that patients with diabetes receive the same level of foot screening and care, regardless of where they receive treatment. This consistency is important for ensuring that patients receive the best possible care and that clinicians follow evidence-based practices (Maxim et al.,2014).

- Risk Stratification: Standardized guidelines can help identify patients who are at high risk of developing foot complications due to peripheral neuropathy. These patients can then be targeted for more tailored foot care interventions, such as advice on foot care, appropriate footwear and regular foot assessments (International Diabetes Federation, 2015).
- Timely referrals: Standardized guidelines can help clinicians determine when to refer patients with peripheral neuropathy to specialists for further examination and management. Timely referral can ensure that patients receive appropriate care and prevent complications from worsening (Formosa et al.,2016).
- Cost-effective care: By identifying and treating early stages of peripheral neuropathy, one can help prevent costly foot complications, such as hospitalizations, surgeries and amputations. This can lead to significant deduction in cost for both the patients and healthcare system (Barshes, et al., 2013).

The variations between guidelines are dependent on location, varying healthcare systems, availability of trained healthcare workforce and specialization in the diabetic foot management (NICE, 2015).

A study conducted by Formosa et al. (2016), has highlighted the importance of regular diabetic foot screening which includes vascular and neurological assessment together with an extensive dermatological and biomechanical examination within Primary health care clinics. This will help in the management associated with foot deformities / posture and to reduce the risk of foot complication as well as classifying the patient’s foot risk category (Fig. 9). Screening guidelines should incorporate easy to use inexpensive tools to successfully be implemented in a clinical setting.

Risk category	Definition	Treatment recommendations	Suggested follow-up
0	No LOPS, no PAD, no deformity	• Patient education including advice on appropriate footwear.	Annually (by generalist and/or specialist)
1	LOPS ± deformity	• Consider prescriptive or accommodative footwear. • Consider prophylactic surgery if deformity is not able to be safely accommodated in shoes. Continue patient education.	Every 3–6 months (by generalist or specialist)
2	PAD ± LOPS	• Consider prescriptive or accommodative footwear. • Consider vascular consultation for combined follow-up.	Every 2–3 months (by specialist)
3	History of ulcer or amputation	• Same as category 1. • Consider vascular consultation for combined follow-up if PAD present.	Every 1–2 months (by specialist)

Figure 9- Risk Classification following a Comprehensive Foot Examination

(Boulton et al.,2018)

Furthermore, multidisciplinary management is important to assure that patients with diabetes are provided optimal care. Foot screening guidelines should focus on health education, routine diabetic foot assessments and biomechanical examination to detect at an early stage any foot abnormalities that may lead to complication (Formosa et al., 2013).

For this reason, it is important to formulate standard screening recommendations to guide the clinician with direct referral, required management and the frequency of diabetic foot screening review (Schaper et al.,2020).

Multiple organizations such as the World Health Organisation (WHO) and the International Diabetes Federation (IDF) made various recommendations for reliable screening guidelines and management of the diabetic foot. Their objective was to decrease or prevent complications such as amputations by half which reduces patient’s morbidity and health cost (NICE, 2015).

Screening modalities are chosen based on evidenced research and should be pertinent to the target population’s characteristics (Kuhnke et al.,2013).

To-date various screening guidelines have been made by multiple organizations aiming to provide the best method to detect neuropathy in its early stages. However there is a lack of consensus between guidelines when it comes to identifying which tool is ideal for accurate diagnosis. Therefore further research is essential in determining which available modalities are best used in a clinical setting (Azzopardi et al., 2018). Every guideline, grades its recommendation depending on the quality of evidence and its strength. Different guidelines have shown inconsistencies when grading their recommendations as they make use of different systems. This further challenges researchers and clinicians to understand the grading system being used (Formosa et al.,2016).

The National Institute for Clinical Excellence of England and Wales (NICE) suggests assessing loss of protective sensation by using the 10g monofilament or testing for vibration perception by using the neurothesiometer or conventional 128Hz tuning fork. It also recommends that the 10g monofilament is replaced after using it on 10 patients to recover the buckling strength (National Institute for Health and Care Excellence, 2019).

The examination guidelines recommended by NICE showed grade C evidence from descriptive non-experimental research studies or extrapolated suggestions from meta-analysis of randomized controlled studies. Therefore, these foot screening guidelines when applied to their target population indicate that such recommendations have poor quality clinical studies (Formosa et al., 2016). The recommended vibrating screening tools were graded A III, indicating that the evidence was obtained from well-thought non-experimental research study and such a suggestion had an overall good-quality literature without extrapolation (Formosa et al.,2016).

Deutsche Diabetes Gesellschaft (DDG) of Germany is another guideline which recommends that the 10g monofilament and the conventional 128Hz tuning fork is used for foot sensation vibrating testing. Unfortunately, the guideline publisher did not provide any grading or evidence for these suggestions (Ziegler et al.,2014).

The Australian National Health and Medical Research Council (NHMRC) recommend the use of the Neuropathy disability score and carrying out ankle reflexes and other sensory tools such as vibration, temperature perception and pinprick. The tuning fork and the neurothesiometer were the two modalities suggested for vibration testing, whilst the 10g monofilament was suggested for touch sensitivity (National Health and Medical Research Council, 2015). This guideline was given an evidence level of grade C as per NHMRC grading system which indicates that the evidence provided is satisfactory but should be considered with caution (Lesse et al.,2006).

Another guideline is from the Canadian Diabetes Association (CDA) suggests the use of the 10g monofilament on the distal-plantar aspect of the feet for the detection of peripheral neuropathy (Booth et al.,2013). It was graded a level D as it lacked various principles which include the independent interpretation of both the test results and diagnostic standard without repeatable definition, as well as inadequate population selection where 50 patients had diabetes mellitus and another 50 were without (Feng et al.,2011).

The Scottish Intercolleglate Guidelines Network (SIGN) proposes the use of the 10g monofilament and the Neuropathy Disability Score as well as the neurothesiometer if further assessment is required (SIGN, 2013). SIGN were graded a 2++ level of evidence results from a study of strong design; although, multiple inconsistencies were noted in the provided findings (Formosa et al., 2016).

The Institute for Clinical Systems Improvement in the USA endorses the use of the 5.07g Semmes- Weinstein monofilament or testing for vibration perception with the 128Hz Tuning fork on the dorsal inter-phalangeal joint of the hallux for any signs of sensation deficit. This guideline has been deemed to have low quality evidence and is suggested that further research is essential to strengthen the thought behind this proposal (Redmon et al.,2014).

On the other hand, the American Diabetes Association (ADA) advocates using the 10g monofilament along with whichever vibration test required such as the 128Hz Tuning fork, ankle reflexes, pinprick sensation and/or neurothesiometer. This guideline obtained a grade B level of evidence as it supports well-conducted cohort research studies (ADA, 2014). Although, this guideline's publisher failed to provide any reference to the evidence with only citing Boulton et al. (2008) which was still not sited next to the recommendation (Formosa et al.,2016).

The International Working Group on the Diabetic Foot (IWGDF) suggests annual foot screening to assess for any symptoms related to peripheral neuropathy as well as to determine whether the foot has become at-risk of developing ulcerations. This organization recommends that the clinician makes use of the 10g monofilament to assess pressure perception and the 128Hz Tuning fork for vibration testing. Furthermore, it is also suggest that when the mentioned screening tools are not available, the light touch test can be carried out. Also, the IWGDF recommends using the risk stratification category system to help determine the appropriate screening frequency and management for prevention (IWGDF, 2023).

Another organization, the International Diabetes Federation (IDF) also favors using the 10g monofilament, 128Hz tuning fork and the pin-prick test for the detection of neuropathy.

However, it also recommends the option of using the neurothesiometer for quantitative assessment where if the result is more than 25 volts, the patient is deemed to have a higher risk of ulcer formation (Formosa et al.,2016). This guideline recommendation was well-evidence based however it still remains ambiguous to where it was originated from. It only provided citations from other guidelines such as the Bakker et al., (2012), SIGN (2013), Bowering & Embil (2013); ADA (2014), NICE (2014) and NHMRC (2015).

Finally, the New Zealand Society for the Study of Diabetes (NZSSD) has a Podiatry Special Interest Group which suggests using the Semmes-Weinstein 10g monofilament, Conventional 128Hz Tuning fork and the neurothesiometer for diabetic sensory examination (Garrett, et al., 2014). This guideline was given a Grade C level of evidence as its findings were based off low-quality observational studies or extrapolated evidence. Furthermore, the guideline publisher did not provide any reference to their evidence (Garrett, et al., 2014).

A study conducted by Formosa et al., (2016), analysed the existing diabetic foot screening guidelines to evaluate the recommendations for screening peripheral neuropathy in patient with diabetes mellitus. A total of ten guidelines were evaluated where the authors noted that most organizations recommended the use of the 10-g monofilament in combination with vibration perception testing such as the 128-Hz tuning fork or biothesiometer/neurothesiometer. While all guidelines emphasized the significance of peripheral neuropathy screening in order to detect the insensate foot, the evidence supporting this recommendation varied considerably when assessed by respective organizations. This lack of consistency is perplexing given that the purpose of evidence-based guidelines is to enhance the quality of healthcare by providing recommendations for optimal screening and treatment methods to aid in clinical decision-making and improve patient care.

Similarly, a review by Chicharro-Luna et al. (2020) compared different guidelines for screening of DPN and found significant variation in recommendations. The authors also highlighted the need for standardized guidelines to improve clinical decision-making and reduce variations in practice.

Furthermore, a study conducted by Mclllhatton et al. (2021) examined guidelines from different professional organizations and found significant differences in screening recommendations, including the test type and protocol as wells as the frequency of screening. The authors also emphasized on the importance of establishing evidence-based guidelines for DPN screening to ensure optimal patient outcomes.

All of the guidelines reviewed in this chapter have highlighted the importance to include sensory screening to assess the presence of peripheral neuropathy. Many similarities with regards to screening tools were noted however the grading level of evidence varies between one another (ADA, 2014).

There were also inconsistencies with the systems used for grading causing poor standard of transparency. The lack of agreement between recommendations contradicts the purpose behind setting such guidelines, to instruct clinicians on which screening tools and treatment plans are best to be utilized in standardized care (Formosa et al.,2019).

Throughout the years, the advancement in technology of screening modalities, the treatment outcomes and disease progression, has led to ongoing research with regards to the ideal clinical tools for the diagnosis of foot complications within a clinical setting as well as continuously creating more accurate guidelines. These advancements which occur through evidence-based research will reduce the risk of ulcer formation and life-altering foot conditions as well as improving the individual’s quality of life (Formosa et al.,2016).

Therefore, overall, standardized foot screening guidelines for DPN are important to ensure that patients with diabetes receive high-quality foot care and that healthcare providers follow evidence-based practices. By following these guidelines, healthcare providers can identify patients at risk, implement appropriate interventions, and prevent serious foot complications (Mclllhatton et al.,2021).

2.9 Local Context- Assessment of Peripheral Neuropathy

In Malta, standard operating procedures (SOP) were implemented to provide podiatrists with a set of guidelines to follow during diabetic foot screening for peripheral neuropathy in a primary care setting. Clinicians generally utilize the 10-site method utilizing the Semmes-Weinstein 10g monofilament and the conventional 128Hz tuning fork when conducting neuropathy screening tests. The frequency of diabetic foot screening review is determined by the outcomes of these two neuropathy screening modalities as well as vascular status of the patient to be able to categorize the foot as low risk or high risk. Furthermore, general foot care advice and skin inspection is carried out routinely especially in individuals diagnosed with peripheral neuropathy. The podiatrist may also work along with a multi-disciplinary team and might refer patient for further management such as at the Orthotics and Prosthetics Unit for therapeutic footwear and/ or the diabetologist to control glucose levels (Mizzi et al.,2021).

Locally, diabetes foot screening is also affected due to the lack of universal guidelines on the ideal devices required to correctly diagnose peripheral neuropathy. Moreover, the International Diabetes Federation has highlighted the importance of formulating and implementing culturally proficient diabetic foot screening guidelines in the aim to decrease foot complications and amputations nationally (Formosa et al.,2019).

The Maltese National Strategy for Diabetes was set in place from 2016 till 2020. It intended to create measures in preventing diabetes, diversify treatment plan options and improve management of diabetes with the goal to delay or prevent onset of complications (Ministry of Energy and Health, 2014).

Although this strategy was a good initiative, it unfortunately lacked in recommending the need of formulating local foot care screening guidelines. It also did not develop local pathway guidelines for continued care within all clinical settings especially in general and emergency medical care. This would have ensured that proper assessments and appropriate referrals to podiatry services are carried out by trained health care professionals which would then decrease the risk of complications within the diabetic population as well as reduce hospital costing (Formosa et al.,2016).

A study conducted by Formosa et al. (2019), indicated that there are three noticeable barriers which are contributing factors in the implementation of local diabetic foot care guidelines. These include organizational factors, healthcare professional factors and patient factors. It was noted that inadequate human and financial resources and the lack of a diabetes register system is hindering improved care and therefore causes an organizational barrier. These shortcomings can lead to no contact between the health care professional and the individual with diabetes therefore making them unaware of essential foot screening examinations carried out in health care settings. This can also delay prevention and early diagnosis of foot complications arising from diabetes.

A factor noted relating to healthcare professionals is the excessive work load in the clinic affecting level of care, time constraints and also limited access to communication within a multidisciplinary team. Moreover, the absence of local foot screening guidelines makes it more difficult in overcoming this barrier (Formosa et al.,2016).

Another mentioned factor which was highlighted in this study was pertaining to patients. It was indicated that the lack of motivation, education, concordance and cultural traditions from patients are a barrier in the current care (Formosa et al.,2016).

The three factors mentioned above further underlines the importance of formulating national strategic diabetic foot screening guidelines and pathways in ensuring optimal patient care (Formosa et al.,2016).

Local foot screening guidelines can be attained by an experienced inter-professional team who supports the system, where they also collaborate between primary care and other specialists when required (Kuhnke et al.,2013).

In addition, it is important to note that foot screening guidelines not only contribute to ensuring patients receive optimal care but also play a crucial role in managing clinical workload and improving access to healthcare services (Formosa et al.,2016).

2.10 Subjective vs. Objective Screening tools

In the field of medical diagnostics, subjective and objective tools are utilized to assess various conditions. However, both subjective and objective screening tools have their strengths and weaknesses when it comes to detecting DPN (Carmichael et al.,2021).

Subjective screening tools are often used to identify patients at risk of developing peripheral neuropathy. Some modalities such as questionnaires and pain scales ie DNS are based on the patient's self-reporting of symptoms. However, these tools are limited by the potential of patients to under-report or over-report symptoms, leading to false positives or false negatives (Gewandter et al.,2016). Furthermore, some of the screening tools also rely on the patient's subjective perception and interpretation of sensations such as vibration or pressure as well as their understanding of the screening process. The inability to properly comprehend the procedure can result in inaccuracies in the obtained results (Won & Park, 2016).

A common example of a subjective screening tool is the Semmes-Weinstein 10-g monofilament test, which relies on the patient's ability to perceive pressure on the skin. Other subjective screening tools of DPN include the 128-Hz TTF, neurothesiometer and the 128-Hz ETF (O'Brien & Karem, 2014); (Brown et al.,2017).

One of the strengths of subjective screening tools is their ease of use, availability and low cost. Additionally, they can be helpful in identifying patients who may need further testing or evaluation (Jimenez & Gili Rius, 2022).

However, in subjective tests, their reliability and validity may be influenced by factors such as patient cooperation, language barriers and cognitive impairment which can limit their usefulness in certain populations (Smith et al.,2013).

The interpretation of test results in a meaningful manner often requires clinical information. While clinical information is subjective, it is still necessary for a comprehensive understanding. Tests utilized in clinical settings should be guided by the subjective clinical examination, as without it, the results may lack context and meaning. Subjective aspects are inherently open to biased interpretation. Therefore, it is crucial to recognize that clinical evaluation remains essential, and the quality of that evaluation is paramount for accurately interpreting any test result (Spaeth et al.,2014).

Objective screening tools for DPN include a variety of methods such as nerve conduction studies (NCS) and skin biopsies. These tests have the advantage of providing objective, quantitative measurement of nerve function and can help identify DPN at an earlier stage. It also helps detect the extent and severity of peripheral neuropathy as well as monitor its progression and evaluate the effectiveness of treatment (Sharma et al.,2015). Therefore, unlike in subjective tests, the results obtained from objective screening tools are not dependent on patient-reported symptoms and are not subject to interpretation by the clinician (Carmichael et al.,2021).

For instance, studies have shown that NCS can be able to detect subclinical neuropathy, which can lead to early intervention and prevention of more severe complications. However, these tests can be costly and time-consuming and their interpretation requires specialized expertise. Also, some of these tests such as skin biopsies are invasive and may not be well-tolerated by patients or recommended for screening purposes (Carmichael et al.,2021).

Furthermore, it is suggested that the clinician does not solely rely on objective testing for diagnosis but also consider the patient’s medical history and physical examination to ensure proper assessment (Carmichael et al.,2021).

A combination of both subjective and objective screening tools may provide the most comprehensive assessment of DPN, as by using both methods, clinicians can better detect DPN and provide appropriate interventions to prevent or slow its progression (Medrano & del Mar Gili, 2022).

2.11 Peripheral Neuropathy Screening Modalities

DPN substantially increases the risk of forming ulceration, leading to amputations and affecting the individual's quality of life. It has been estimated that half of the diabetic population present with peripheral neuropathy and may present with unnoticeable symptoms. Therefore, this further underlines the importance of finding a sensitive and specific tool which is inexpensive and not time consuming in diagnosing peripheral neuropathy (Jayaprakash et al., 2011); (Pop-Busui et al.,2017).

It is essential that as part of the diabetic foot screening, the sensory nervous system is assessed to determine any loss or disruption of sensation in the dermatomes being analysed (Chung et al.,2014). Therefore, clinicians use diagnostic methods with high sensitivity and specificity to ensure their usefulness and accuracy as compared to the reference standard. Sensitivity in a diagnostic tool can be described as having a high probability of identifying a true positive of the disease. Whereas, specificity of a diagnostic tool refers to its ability to accurately identify individuals who do not have the condition being screened for. It measures the proportion of true negative results among individuals who actually do not have the condition. In other words, it quantifies the tool's capacity to rule out the presence of the condition in healthy individuals (Travevthan, 2017).

There are various valid screening methods available for the diagnosis of peripheral neuropathy, however there is limited research on which tools can be used to accurately detect peripheral neuropathy in its early stages (Papanas & Ziegler, 2012). Furthermore, it is important to note that each tool possesses its own distinct set of advantage and disadvantages (Fig. 10) which consequently leads to a need for careful consideration and evaluation (Carmichael et al.,2021).

		Nerve Fibers Assessed	Advantages	Limitations
Symptoms and Signs	**Questionnaires**	Large (Aβ-fibers) and Small (Aδ and C-fibers)	Easy to administer. Used for monitoring symptoms	Lack of Sensitivity, accuracy and reproducibility, Subjective
	NDS	Large (Aβ-fibers) and Small (Aδ and C-fibers)	Does not require specialist equipment, Assesses large and small-fiber function	Not sensitive or reproducible, Low correlation with small fiber quantitative tests
	10-gram Monofilament	Large (Aβ-fibers)	Simple, quick and inexpensive	No standardization of methods. Cannot detect early neuropathy
	Ipswich Touch Test	Large (Aβ-fibers)	Simple. Requires no specialist equipment .Can test at home	Can only detect advanced neuropathy
	QST (Thermal and Vibration thresholds)	Large (Aβ-fibers) and Small (Aδ and C-fibers)	Measures small and large fiber function .Good repeatability	Unable to differentiate between peripheral and central abnormalities High inter-operator variability
Large FiberTests	**DPNCheck**	Large, sural nerve (Aβ-fibers)	Quick, Easy to perform,Good sensitivity (92-95%) compared to NCS	Relies on the accessibility of sural nerve . Validation studies had small patient numbers
	NCS	Large (Aβ-fibers)	A sensitive measure of large nerve function ,Reproducible	Doesn't assess small fibers, Uncomfortable, Does not assess early neuropathic changes
Small Fiber Tests	**Skin Biopsy (IENFD)**	Small (C-fibers)	Gold standard for SNF, Quantitative,Good sensitivity,Detects early nerve changes	Invasive, Risk of infection, Repeatability, Requires trained personnel and special labs
	CCM	Small (Aδ and C-fibers)	Non-invasive,Good reproducibility, Rapid and objective	Relatively Expensive,Requires specialist equipment and personnel,manual analysis is time-consuming
AutonomicTests	**Neuropad**	Small (C-fibers)	Can be self-administered,suitable for screeningNon-invasiveGood sensitivity	Varied interpretation of the results
	Sudoscan	Small (C-fibers)	Non-invasive,Easy to perform	Unclear if measuring sudomotor function Variable specificity (53-92%)
	QSART	Small (C-fibers)	Sensitive for SFN (82%) Gold standard for measuring sudomotor function	Time-consuming, Requires specialist equipment and trained personnel Uncomfortable

IENFD, Intra-epidermal nerve fiber density; NCS, Nerve conduction studies; QSART, Quantitative sudomotor axon reflex test; CCM, Corneal confocal microscopy; NDS, Neuropathy disability score; QST, Quantitative sensory testing; SFN, Small fiber neuropathy.

Figure 10- Diagnostic tools available for assessing DPN

(Carmichael et al.,2021)

DPN progresses with time and it usually has a subclinical presentation in its early stages. Unfortunately, research is lacking when it comes to which diagnostic modalities can detect subclinical changes. Most studies focus on using expensive and non-portable nerve conduction testing for early accurate diagnosis. Although, nerve conduction testing exceeds other screening tools, the majority of clinics are not equipped with such a modality. This is because nerve conduction testing is time-consuming and its running costs are expensive. Therefore, for the best positive outcomes, more research is required to determine which readily available, portable inexpensive practical tool is best to use (Mustafa et al.,2012).

There are multiple screening tools such as questionnaires and sensory tools that have been deemed reliable and valid by research studies. Some examples of these tools include the Michigan Neuropathy Screening Instrument, Semmes-Weinstein 10g monofilament assessing pressure sensation and the 128Hz tuning fork for vibration perception (Richard et al.,2012).

The Michigan Neuropathy Screening Instrument (MNSI) can be used to assess the presence of peripheral neuropathy by using a 15-item questionnaire which only requires yes/no responses. It was deemed by multiple studies to be reliable and valid in detecting DSPN. However, studies suggest the MNSI should not be used solely but with conjunction to other screening modalities especially when peripheral nerve involvement is suspected (Mete et al.,2013). Its limitations are due to patient subjectivity to symptoms as it completely relies on the individual's perspective therefore producing low diagnostic accuracy (Himeno et al.,2019).

Another questionnaire is the Diabetic Neuropathy Symptom Score (DNS) consisting quantitative scoring system assessing any neuropathy related symptoms currently experienced by the patient. Meanwhile, the Diabetic Neuropathy Examination (DNE) quantifies the extent of neuropathy through the use of questionnaire. If the patient scores less than three then the presence of peripheral neuropathy is evident (Yang et al.,2018).

In a research study conducted by Jayaprakash et al. (2011), the DNE and DNS scoring system obtained similar specificity and sensitivity results. Although, the results obtained for sensitivity varied when compared with other studies as such questionnaire is subjective.

Moreover, the DNE scoring system was deemed to be more time consuming when compared with other screening modalities like the 10g monofilament and the 128Hz Tuning Fork (Mythili et al.,2010).

In a clinical setting, both pressure sensitivity examination and vibration perception testing are mostly used for the detection of DPN. The examination of pressure sensitivity is most commonly assessed using the Semmes-Weinstein 10g monofilament (Yang, et al., 2018). On the other hand, vibration testing is frequently assessed using the 128Hz tuning fork as it is less costly than other vibration devices available although other modalities have also been documented to becoming more popular in using along with the tuning fork (Raymond et al.,2020).

Both of these modalities are subjective screening tools since they depend on patient feedback. Moreover, variations in user technique, testing site selection and material fatigue further contribute to the inaccuracy of these tests (Lavery et al.,2012); (O'Brien & Karem, 2014). Some authors advocate the use of the 128Hz traditional tuning fork over the 10g monofilament, as vibration perception is thought to be affected at an earlier stage (Edmonds, 2020). Furthermore, some researchers have reported ambiguous clinical efficacy with the 10-g monofilament test (O'Brien & Karem, 2014).

Many studies have determined that these two screening tools were deemed to be good predictors in establishing the risk of individuals in developing foot complications such as ulcerations. However, some studies suggest that such screening tools were able to detect a later stage of peripheral neuropathy (Ramanathan et al.,2021).

The posterior nervous column is usually assessed for vibration perception as it responsible for both proprioception and vibration (Spruce, 2012). The testing area for vibration sensation is usually the hallux since many research studies have confirmed it to be the most appropriate location for testing (O’Brien & Karem, 2013). It is essential that clinicians test for the loss of vibration sensation as this stimulus is usually affected in the initial stages of peripheral diabetic neuropathy (Edmonds, 2020).

The International Diabetes Federation (IDF), International Working Group on the Diabetic Foot (IWGDF) and the American Diabetes Association (ADA) are three major international organizations that develop widely used guidelines for diabetic foot assessment, diagnosis and management (Mclllhatton et al.,2021). These organizations recommend variations of the following non-invasive clinical tests: 10g monofilament, 128Hz tuning fork, light touch test, temperature perception, neurothesiometer, pinprick, proprioception and ankle reflexes (Ibrahim, 2017); (Pop-Busui et al.,2017); (Schaper et al.,2020).

The ADA also suggests the use of Timed vibration testing (TVT) when assessing patients for peripheral neuropathy. The O'Brien 128Hz Electronic tuning fork (ETF) aligns with these recommendations (O'Brien & Karem, 2022).

In addition, the ADA also suggests that nerve conduction studies (NCS) or electromyography (EMG) may be utilized when there is suspicion of nerve damage in patients with diabetes (American Diabetes Association, 2023). In fact NCS is known to be preeminent methods in identifying early non-symptomatic diabetic peripheral neuropathy. However, using such diagnostic methods for screening is impractical, expensive, has restricted availability and is performed by a specialized practitioner (Sharma et al.,2015). Moreover, the need for additional quantitative screening tests that are easily available and suitable for use in a clinical setting is underscored by this. The NC-Stat® DPN Check® is a quick user-friendly quantitative nerve conduction screening tool that has been demonstrated to have a good correlation with standardized NCS, as well as being considered sensitive and specific for peripheral diabetic neuropathy (Chatzikosma et al.,2016); (Shibata et al.,2019).

Therefore, for the purpose of this research study, the subjective screening modalities which include the 128- Hz TTF, Neurothesiometer, 128-Hz ETF and Semmes-Weinstein 10g monofilament were utilized and compared with the NC-Stat® DPN Check® which is an objective nerve conduction tool.

2.11.1 Semmes-Weinstein 10g Monofilament

The 10g monofilament (Fig. 11) is a simple cost-effective subjective clinical screening tool widely used as part of diabetic foot screening to assess pressure sensitivity. The tool consists of a nylon filament with a constant buckle of a 10-gram force and is applied perpendicularly in multiple sites on each foot. The patient acknowledges every time the sensation is felt with a ‘yes’ reply. If patient fails to acknowledge sensation than the site will be interpreted as insensate to the stimulus. Therefore, if one site is not felt, the individual is considered as having loss of large fiber nerve function and an increased risk of developing ulceration (Spruce & Bowling, 2012); (Al-Muzaini & Baker, 2017).

Figure 11- Semmes-Weinstein 10g Monofilament

(Rainier Medical, 2018)

If excessive force is applied, the nylon monofilament will deform irreversibly causing it to produce inaccurate results. This is due to the increased plasticity of the filament when repetitive loading occurs, alternatively causing lower bending forces. Therefore, it is recommended that clinicians replace the nylon monofilament following this incidence (Lavery et al.,2012)

Moreover, to ensure that the 10g force is being applied, the monofilament should rest following 10 tests and changed after 6 months of use. Also, the clinician should avoid applying the monofilament on calluses, scarring, oedema and indurated areas as this may affect the result obtained from the 10-g monofilament (Al-Muzaini & Baker, 2017).

The sensitivity and specificity of the 10g monofilament test have shown significant variability across different studies, likely due to the lack of standardization in its use (Gill et al.,2014). There is no agreement on the number and location of sites to be tested, or which gold standard tool should be used to compare and determine the sensitivity and specificity of the 10g monofilament test. Several studies have utilized clinical testing as the reference or gold standard, whereas others have used biothesiometer or thermal testing (Al-Geffari, 2012); (Pourhamidi et al.,2014).

Other factors that can affect the results obtained from this test include lack of blinding, subjectivity of the test, variations in sole thickness among different ethnic populations and environmental factors such as filament ageing and durability (Gill et al.,2014); (Al-Muzaini & Baker, 2017).

These inconsistencies make it difficult to compare results between studies since there is a considerable variability in the sensitivity and specificity acquired from the monofilament test (Baraz et al.,2014).

A control study conducted by Perkins et al. (2001), showed that the 10g monofilament has a variable sensitivity of 77% and a specificity of 96% respectively. However, a systematic review conducted by Dros et al. (2009), demonstrated wide variation in the sensitivity and specificity of the monofilament test across different research studies, where the sensitivity of the test ranged from 41% to 93% while the specificity ranged from 68% to 100%. These variances may be attributed to dissimilarities in the populations studied, different method application and interpretation of the test. Based on their analysis, it is challenging to ascertain the diagnostic utility of the monofilament testing for DPN screening, primarily because of the lack of studies utilizing standardized techniques and proper methodologies. It has been suggested that the monofilament testing should not be utilized as a sole diagnostic tool and should be complemented with other clinical testing. In cases of uncertainty NCS should be performed to better establish the presence of peripheral neuropathy (Dros et al.,2009).

Gin et al. (2008) has also reported that the 10g monofilament is highly limited by the false negative results obtained.

There is also disagreement regarding the buckle force to be used for effectively diagnosing peripheral neuropathy. Various studies have tested different buckle force ranging from 2g to 10g monofilaments (Jeng et al.,2000). A study by Nagai et al. (2001), recommended that a range of monofilament with different buckle forces should be employed to identify early stages of peripheral neuropathy however it has shown to be time consuming and impractical in a clinical setting. Another study indicated that all participants without diabetes mellitus were able to perceive the 10g monofilament but a percentage reduction was noted as the buckle force applied decreased therefore increase the risk of a false result (Thomson et al.,2008). Additionally, a study conducted by Saltzman et al. (2004), states that a 4g buckle force is clinically more superior than a 10g force due to higher sensitivity.

The lack of agreement regarding the sites required to be tested by the 10g monofilament as stated above is another factor which has caused variability between studies. There is minimal evidence in stating which of the 3 site, 4 site or 10 site method on each foot is valid. Therefore, this makes it difficult for clinicians to choose which method to utilize (Baraz et al.,2014). Zhang et al. (2018), has also showed that there were no significant differences between the three methods and therefore recommended on using the 3-site on each foot for a simpler and less time-consuming test. However, many clinicians still make use of the 10-site method, therefore for this reason it was the method chosen for this research study.

The existing literature does not provide a clear consensus on the specific number of insensate sites when utilizing a 10g monofilament to categorize the risk of developing ulceration. In fact, it was noticeable that when the criteria of negative response sites was increased from one to four to classify a patient at risk of neuropathy, a decline in sensitivity was noted from 86% to 65%, while the specificity increased from 58% to 71% respectively (Miranda-Palma et al.,2005) (Zhang et al.,2018).

Although there is a lack of consensus regarding the best protocol to be employed with monofilament testing, it is still considered to be a common tool utilized for the detection of severe neuropathy with the risk of developing ulceration (Tan, 2010). In fact, an abnormal monofilament test is linked with a relative risk of 15% for the development of foot ulceration or lower limb amputation over a 3-year period (Burgess et al.,2021).

Owing to these variations and factors, it is not unexpected that there is dissimilarity between studies with regards to the monofilament test's reproducibility which ranges from poor to good (Lai et al.,2014); (Bishop & Poole, 2022). Additionally, there are variations in its diagnostic accuracy, with sensitivity ranging from 6% to 93.1% and specificity from 68% to 100% (Blankenburg et al.,2012); (Hirschfeld et al.,2015).

Despite some questioning the efficacy of monofilament testing as a tool for detecting peripheral neuropathy, other studies have suggested that it is a reliable and repeatable modality (Young et al.,2011); (Mclllhatton et al.,2021). A study conducted by Lanting et al. (2020), stated that the monofilament had a moderate inter-rater reproducibility indicating that different independent investigators mostly obtained the same results (Lanting et al.,2020).

Although numerous studies have indicated that the monofilament test may only identify DPN at an advanced, irreversible pre-ulcerative stage it is still recommended for clinical use in by multiple guidelines. Moreover, most of these guidelines suggest that in a clinical setting the 10g monofilament should not be used solely but with a variety of screening modalities to obtain better results (Burgess et al.,2021).

2.11.2 128-Hz Traditional Tuning fork

The 128-Hz Traditional Tuning fork (TTF) is an inexpensive, easy-to-use and readily available screening tool commonly used for the assessment of vibration perception in a clinical setting. Over the years, there has been extensive research on the various methods of use and modifications of the tuning fork. Nevertheless, in a clinical setting the 128-Hz TTF is generally recommended in foot screening guidelines (Yang, et al., 2018).

The TTF comprises of two tines, a stem and a baseplate (Fig. 12) that when struck produces a frequency. It is a simple test where the examiner strikes the tines causing vibration without the presence of audible humming (Schaper et al.,2016).

Figure 12- 128-Hz Traditional Tuning Fork

(Saris, 2015)

There is an array of literature describing the numerous testing methods available when using the 128-Hz TTF. However, all these different protocols can be generally split into two testing categories which are the On-Off method or timed assessments. The On-Off method is when the patient simply states whether the vibratory stimulus was perceived on the area being tested following the placement of the TTF (Schaper et al.,2016); (Raymond et al.,2020).

On the other hand, timed assessments rely on the principle that the amplitude of the stimulus diminishes gradually over time and considers the duration for which a patient can detect the vibration of the tuning fork. The outcome measure is determined by comparing the duration of vibration sensation felt by the patient with that of the clinician. Therefore, the patient informs the examiner when the stimulus is no longer perceptible and the clinician places the baseplate on their own thumb. If the vibration continues for an additional 10 seconds, the patient is classified as having diminished sensation (Barohn & Amato, 2013); (Al-Muzaini & Baker, 2017).

A variation of the timed assessment 128-Hz TTF is the Rydel-Seiffer tuning fork which has a graduated scale therefore providing a quantitative outcome measure. The result is displayed on the fork at the point of cessation of vibration sensation. Some researchers have deemed the graduated tuning fork as being more accurate than the 128-Hz TTF although both have shown limited sensitivity (Lai et al.,2014).

Both the graduated and non-graduated tuning fork resulted to have moderate intra-rater reliability. The graduated tuning fork demonstrated to have a slightly higher inter-rater reliability when compared with the conventional method. However the non-graduated tuning fork still obtained substantial inter-rater reliability (Lanting et al., 2020).

The on/off method and the timed vibration testing (TVT) are both methods recommended by various diabetic foot screening guidelines. For instance, the ADA recommends the TVT method for assessing vibration perception (O'Brien & Karem, 2022). However, there is no agreement on which method is best to be utilized when assessing with the 128Hz TTF. The variations in the methods used create lack of standardization between researchers and clinicians (Brown et al.,2017).

Furthermore, most clinicians opt to test the hallux with the 128-Hz TTF although some screening protocols also recommend testing the malleoli. Research has demonstrated that patients who felt the vibration stimulus on the hallux had also felt the sensation on the malleolus. On the other hand, individuals who were able to feel vibration sensation in the malleolus might not necessarily feel the stimulus on the hallux. Therefore, this indicates that the test should primarily be conducted on the hallux (Gin et al., 2008); (Bakker et al.,2011); (Takahara et al.,2014).

A study conducted by Singh et al., (2005) indicated that the 128-Hz TTF resulted in having 53% sensitivity and 99% specificity. Another research study by Jayaprakash et al. (2011) stated that a higher sensitivity percentage of 62.5% was found whilst the specificity resulted slightly lower than previous studies at 95%. Furthermore, a study conducted by Al-Geffari (2012), produced similar sensitivity percentage of 72.5% as well as an accuracy percentage of 81.4%. This researcher suggested that the 128Hz tuning fork is more sensitive than the Semmes-Weinstein monofilament test and the ankle reflex test, where the possible reason being that the vibration sensation is initially lost in the early stages of neuropathy. Another study found that the 128-Hz TTF had a sensitivity of 21% and a specificity of 88% (Lai et al.,2014).

Moreover, many studies have demonstrated a wide range of reliability levels from none to strong when evaluating the inter-rater reliability of the 128-Hz TTF (Arshad et al.,2016); (Lanting et al.,2020). Additionally, two other studies reported weak to moderate intra-rater reliability and weak inter-rater reliability of the TTF (Mclllhatton et al.,2021).

Even though the 128Hz TTF is widely used, researchers have criticized its limitations by questioning the interpretation of results obtained, lack of consensus regarding methodology and having no standardized vibration frequency (Goddard et al.,2018). Furthermore, the results obtained may also be affected by the amount of pressure applied on the testing site and the intensity of the initial stimulus may not be constant and is dependent on the force applied by the examiner (Levy, 2010); (Bishop & Poole, 2022).

When placing the tool on the skin overlying fat or muscle, the vibration is transmitted through a significant volume of tissue resulting in decreased reliability. Additionally, it is crucial for clinicians to ensure that the patient is responding specifically to the produced vibration rather than other sensations such as pressure or sound of the tuning fork itself (Lanting et al.,2020).

These factors, including the potential variations in different areas of application and the inherent difference in vibration strength during each application, contribute to the poor to moderate reproducibility of the test (Lanting et al.,2020). The application of this tool has also been deemed as limited by patient responsiveness and observer technique (Dubey et al.,2022).

Researchers have recommended that relying solely on the 128-Hz TTF for the detection of peripheral neuropathy should be avoided, and instead should be used in conjunction with other available screening tools (Brown et al.,2017). Moreover, numerous international guidelines have suggested using both the Semmes-Weinstein 10-g monofilament for pressure testing and the 128-Hz TTF for vibration perception as part of diabetic foot screening (Richard et al.,2012).

2.11.3 Neurothesiometer

The biothesiometer was initially developed to provide standardized and mechanical vibration perception. However, it was later replaced by the battery-operated neurothesiometer, which operates on the same principle (Dubey et al.,2022).

The neurothesiometer is a user-friendly quantifiable tool to measure vibratory perception threshold. This tool generates mechanical vibration with a constant frequency of around 100Hz, and the vibration amplitude is adjusted manually thorough a knob on the device. This screening modality assesses nerve function by adjusting the voltage using the knob ranging from 0 to 50V. (Dubey et al.,2022).

The device consists of a stylus which generates vibration stimulus at a given frequency (Fig 13). The clinician firmly places the probe perpendicularly on the distal aspect of the patient's hallux and starts to gradually increase the vibration amplitude at a rate of 1V/second (Lanting et al.,2020).

The patient will then notify the clinician when vibration is perceived and the corresponding value is recorded. It is suggested that the test should be repeated three times for a mean value to be calculated (Jayaprakash, et al., 2011); (Lanting et al.,2020). The amplitude obtained will determine the grade of neuropathy as low, moderate or high risk of neuropathy; (Dubey et al.,2022).

Figure 13- Neurothesiometer

(Williams Medical, 2012)

A study conducted by Gin et al., (2008) showed that individuals who were insensate to a vibration stimulus of 25V are seven times more at risk to developing an ulcer within four years than those with a lower sensitivity threshold.

Lanting et al. (2020), reported that the vibratory perception threshold (VPT) has a moderate to substantial intra-rater reliability and a substantial inter-rater reliability. Moreover, this study showed that experienced raters obtained a substantially lower reliability than less experienced clinicians.

Nonetheless, controversy exists regarding the sensitivity and specificity of the VPT due to variations in the choice of gold standard tool used to determine its accuracy. A study by Bracewell et al. (2012), reported that the neurothesiometer obtained a sensitivity and specificity of 80% and 98% respectively. While a study by Pourhamidi et al. (2014), found that the sensitivity and specificity for detecting DPN was 82% and 70% respectively and exhibited considerably lower percentages for the diagnosis of small fibre neuropathy. Researchers argue that small nerve fibers are affected earlier than large nerve fibres in DPN. The study considered the NCS and DNS as the 'gold standard' tool. Another study conducted by Saha et al. (2011), assessed the VPT for the early detection of DPN. The study included 60 diabetic patients, half showing clinical evidence of neuropathy and the other half without clinical evidence of neuropathy based on the MNSI. Among the group with clinical neuropathy, 26.6% showed no neuropathy by the VPT where the discrepancy may be attributed to the subjective nature of the test. Furthermore, the majority of patients with clinical evidence of neuropathy were classified as having neuropathy with VPT.

Moreover, various authors have found the neurothesiometer to be a reliable method for assessing VPT when compared to various tuning fork applications (Baker et al.,2012); (Lanting et al.,2020). However, there is inconsistency in determining the optimal cut-off values for detecting DPN. In various studies, a VPT ≥ 25V was used as one of the diagnostic criteria for DPN in individuals with diabetes (Adams et al.,2019). Though, other studies diagnosed DPN based on a

VPT ≥ 15V as well as other cut-off values ranging between 15V and 25V (Ponirakis, et al., 2020); (Ramanathan et al.,2021). In addition, a study by Javed et al. (2015), found that the 25V threshold exhibited low sensitivity for the early detection of DPN and lowered threshold was preferred. These variations in cut-off values for diagnosis are due to the use of different reference tools in defining DPN such as NCS, NDS or the MNSI (Liu et al.,2021).

Therefore, this means patients with VPT of ≥ 25V should be informed about the increased risk of ulceration and educated on proper foot care practices. Also, patients with VPT values between 15V and 25V, should receive regular follow-up care and also advised on how to best manage their glycaemic levels to potentially delay the progression of DPN (Ramanathan et al.,2021).

A study conducted by Dubey, et al. (2022), took into consideration the significant risk factors associated with DPN. These variables can be utilized to predict the VPT or estimate the predicted VPT along with the confidence interval. The study stated that to facilitate the interpretation of the VPT for clinicians and patients, it was valuable to interpret the VPT prediction based on cumulative risk levels. To address neuropathy classification by the device the initial step involved dividing the data into distinct categories determined by VPT thresholds. Considering the expertise of clinicians in the field of diabetic neuropathy, the dataset was classified into three classes based on VPT measurements namely low risk, medium risk and high risk. The VPT cut-off for these categories were as follows, amplitude of 0V to 20.99V indicate low risk and 21V to 30.99V exhibit medium risk whilst a VPT ≥ 31V are considered as having high risk of neuropathy (Dubey et al.,2022).

The neurothesiometer has its limitations such as the pressure applied on the vibrating probe, limb temperature and site, tactile surface of the skin, patient understanding and psychological factors can confound the results. One can note that most of its limitations are due to the subjectivity of this test (Ramanathan et al.,2021). Moreover, other considering factors which may limit the use of neurothesiometer by researchers were its cost, time taken to carry out the test and size of the device making it not readily available in the majority of clinical settings (Bracewell et al., 2012). It was also noted that the device has its limitations in its reliance on manual observer-dependent operability as well as the vibration intensity produced is limited (Abott et al.,2011); (Dubey et al.,2022).

Despite these drawbacks, the VPT is still regarded as valuable screening tool for DPN and is recommended by various screening guidelines (Ramanathan et al.,2021).

2.11.4 O' Brien 128- Hz Electronic Tuning Fork

The O'Brien 128-Hz Electronic Tuning Fork (ETF) is a relatively new battery operated easy-to-use portable screening device to assess vibration perception threshold. This device electronically mimics the vibration output and decay rate of the traditional tuning fork (TTF). Additionally, unlike the TTF, the ETF uses fixed amplitude of stimulus to initiate each test, while the TTF's amplitude varies depending on the striking force applied by the clinician. It also includes an integrated timer to enable precise and replicable timed vibration tests (TVT), which has been proven to be a validated and effective method for detecting neuropathy (O'Brien & Karem, 2014).

The device (Fig 14) consists of a body from where it is activated and held by the examiner featuring a top button for 'Mode' selection and a 'Run' button to initiate the test. Additionally, the device is equipped with a rounded tip that generates vibration stimulus upon contact with the patient's testing location, while a display monitor shows the elapsed time in seconds upon completion of the test. The hallux timed vibration test scale shown on the device indicates the neuropathy risk level based on the time in seconds obtained (O'Brien & Karem, 2014).

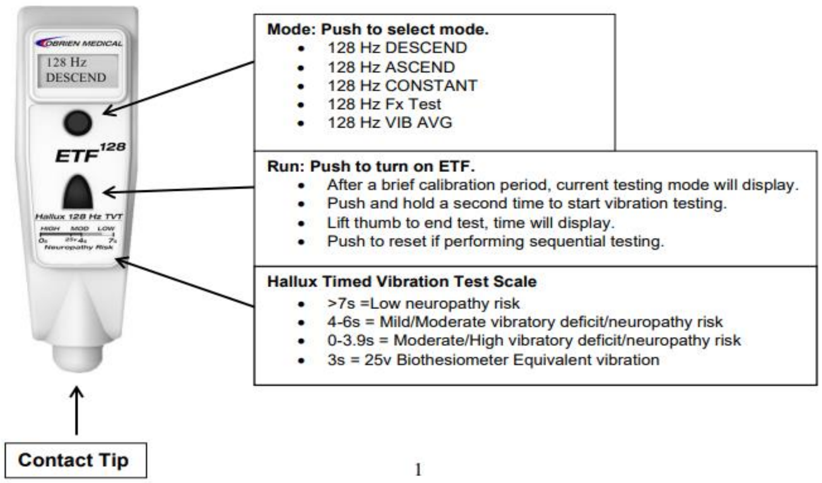


Figure 14- O'Brien 128-Hz Electronic Tuning Fork

(O'Brien Medical, 2022)

The clinicians and researchers can choose from the five output modes available namely the descending mode, ascending mode, constant mode, stress fracture test mode and vibroception averaging mode. The ETF can perform both on-off and timed vibratory assessments which are methods also used in the TTF. These two assessments can be carried out by using the descending mode method and the constant method of the 128-Hz ETF (O'Brien Medical, 2022).

In this research study, the descending and constant methods were employed as they mimic the timed-assessment method and on/off method used by the TTF (Brown et al.,2017).

The 128-Hz ETF descending mode

The descending mode of the 128-Hz ETF produces vibrational output amplitude that gradually decreases from high to zero over a 25- second period, similar to the TTF. The ETF contact tip is placed on the dorsal aspect of the distal phalanx of the hallux, proximal to the nail bed for testing. When the device is placed on the tested area, the examiner initiates the test by pressing the ‘Run’ button, which activates both the vibration and in-built timer. The participant is then requested to indicate the cessation of vibratory sensation by saying ‘stop’, and the examiner stops the vibration and timer by releasing the ‘Run’ button. This point is known as the vibration disappearance threshold (VDT). During the test, the built-in timer starts from 0 seconds up to 25 seconds. A longer duration in seconds during the test indicates a lower risk of peripheral neuropathy. The elapsed time in seconds is displayed on the screen and compared to a reference scale on the device to assess the neuropathy risk of the patient’s limbs (O’Brien & Karem, 2014).

For better results, this process should be repeated bilaterally three times and a mean is taken.

The 128-Hz ETF Constant mode

This mode provides constant vibrational amplitude set to the equivalent of the 25V level in a traditional biothesiometer. This allows users to quickly assess patients for the presence or absence of neuropathy using this established reference standard (O'Brien Medical, 2022).

The patient is asked to close his/her eyes throughout the examination period. The run button is pressed and held to produce the vibration output. The contact tip of the ETF is applied gently, consecutively two times after each other on the patient’s hallux (which is the same area tested in the descending mode) for approximately half a second. The patient is then asked to distinguish which of the two applications was the vibration sensation from the device felt. This procedure should be repeated three times bilaterally for better results (O'Brien Medical, 2022).

This device has been developed as a method for standardized and quantitative assessment of TVT. This approach was tested in a study conducted by O'Brien & Karem (2014), which demonstrated a sensitivity of 0.953 and specificity of 0.761 for detecting neuropathy, using conventional tests as a reference standard.

The ADA also proposes the use of Timed vibration testing (TVT) as part of the diabetic foot screening guidelines. The O'Brien 128Hz Electronic tuning fork (ETF) is aligned with these recommendations for the diagnosis of peripheral diabetic neuropathy (O'Brien & Karem, 2022). The O'Brien 128Hz ETF was also cited in the IDF Clinical Practice Recommendation on the Diabetic Foot (Ibrahim, 2017).

2.11.5 NC-Stat® DPN Check®

Currently, the diagnosis of DPN in a clinical setting is based on patient symptoms, physical findings and subjective examinations. The use of physical signs and symptoms has low reproducibility and accuracy as stated by various researchers, whilst sensory subjective tests are considered more reliable (Shibata et al.,2019). The most accurate way to investigate DPN is by invasive techniques like nerve or skin biopsy which are not suggested for diagnostic use in clinical setting (Carmichael et al.,2021). In contrast, NCS provide an objective non-invasive and reliable method for DPN diagnosis. The NCS are widely regarded as the gold standard diagnostic test for assessing peripheral nerve function due to the high reliability, accuracy, sensitivity, specificity and validation (Shibata et al.,2019).

Regrettably, the widespread implementation of NCS has been hindered by the need for costly equipment and trained specialists. In order to address the lack of accessibility to NCS, a point-of-care nerve conduction device namely the NC-Stat® DPN Check® has been developed (Lee et al.,2014).

The NC-Stat® DPN Check® is a fast non-invasive accurate user-friendly sural nerve conduction test used for the early detection of peripheral neuropathy. Its purpose is to quantitatively measure nerve electrical activity by gathering information about both the nerve structure and function. The device provides the clinician with a quantitative measure of both the sural nerve amplitude potential (SNAP) and a sural nerve conduction velocity (SNCV) of the large myelinated nerve fibers which are standard biomarkers for subclinical and clinical peripheral diabetic neuropathy as well as categorize its severity (Lee et al.,2014).

The device analyses the conduction velocity and the amplitude as both are affected when damage to the nerve fibers occurs. The SNCV denotes the action potential propagation velocity of the nerve fibers. When demyelination of a nerve fiber occurs, the conduction velocity slows down affecting the action potential propagation. Furthermore, nerve axon degeneration reduces velocity conduction which commonly occurs in diabetic microvascular damage. On the other hand, the SNAP denotes the amount of large myelinated axons which are conducting action potential. The decrease in SNAP value occurs when axon degeneration is present which is also commonly present in diabetic complications (Lee et al., 2014).

The NC-Stat® DPN Check® mainly consists of single use biosensors, built-in thermometer, two stimulation probes, LCD monitor to display results and a docking station to transmit data (Fig. 15) (Papatheodorou et al.,2018).

Figure 15- NC-Stat® DPN Check®

(Neurometrix Inc., 2021)

The method for testing is straight-forward and only little training is required to operate and interpret results (Poulose et al.,2015).

Firstly, the patient is asked to lay on the clinic couch in a relaxed recumbent position with the leg being tested on top. The testing site is then swabbed with a preparation pad to remove any oil or excess dead skin. The examiner should have easy access to the lateral malleolus and the Achilles tendon of the leg being tested. Following this, the device is switched on and a disposable biosensor which facilitated nerve conduction is inserted into the port. Once the biosensor is properly placed, the examiner will then select on the LCD monitor which leg will be tested. A small amount of conductive gel is applied on both the anode and cathode stimulation probes. The probes are then aligned to the lateral malleolus where the cathode is placed adjacent to the central prominence of the lateral malleolus and the biosensor placed on the lateral lower 1/3 of the leg. The operator then pushes both the probes and the biosensors firmly down with constant steady force for proper contact (Vinik et al.,2014).

The button on the device is then pressed which will initiate nerve stimulation releasing 100mA of current. An integrated thermometer, accounts for any variances in skin temperature between 23°C to 30°C and the examiner will be notified if the temperature is too low preventing continuation of procedure. If all the parameters required to conduct the test are sufficient, the SNAP and SNCV values are then displayed on the LCD screen in less than a minute. If the individual results in having greater than 4 microvolts (μV) of amplitude and greater than 40 meters per second (m/s) conduction velocity indicate normal limits (Brown et al.,2017).

After obtaining results, the device comes with an interpretation guide that enables the examiner to classify the patient's neuropathy as normal, mild, moderate or severe neuropathy based on the two values provided (Brown et al.,2017). The below table (Fig. 16) indicates how the individual is categorized; the ranges required for classification is further explained in Chapter 3.

Severity	Amplitude	CV
Normal	Normal	Normal
Mild	Normal	Abnormal
Moderate	Abnormal	Normal or Abnormal
Severe	Undetectable	—

Figure 16- Nerve Conduction Reference Ranges

(Neurometrix Inc., 2021)

The test can be carried out unilaterally as DPN usually affects symmetrically however the examiner may decide to repeat the test on the same leg when the results are inconclusive, the second confirmation of result is required or if the patient presented with asymmetrical symptoms. Although, the other leg can also be tested if further confirmation is required. The operator can make use of the same biosensors when testing bilaterally (Neurometrix Inc., 2021).The below table (Fig. 17) shows an example of the test results which may be displayed on the LCD screen with further explanation.

Display Example	Result	Actions
40	Conduction Velocity – meters/second	Record and interpret result.
4•	Amplitude – microvolts*	Record and interpret result.
0•	Undetectable Response; no Conduction Velocity displayed	Record and interpret result.
Pn Pr Sn Lb Ec °C Hd	Test Unsuccessful	Note displayed code and refer to Troubleshooting on back.

Figure 17- LCD Display Example

(Neurometrix Inc., 2021)

The DPNcheck automatically implements a series of quality control procedures with every test to maximize the likelihood of obtaining reliable data. Several of the key quality checks include:

- skin temperature (Neurometrix Inc., 2017)
- to avoid excess amount of conducting gels on stimulator probes (Neurometrix Inc., 2017)
- direct skin contact of the biosensor (Neurometrix Inc., 2017)
- adequate stimulation intensity of up to 70milliamps to overcome oedema, adipose tissue and neuropathy (Neurometrix Inc., 2017)
- average of at least 4 nerve responses (Neurometrix Inc., 2017)
- the nerve response is not contaminated by artifacts such as movement, stimulus or electrical stimulus (Neurometrix Inc., 2017)
- the correct limb is selected on the device (Neurometrix Inc., 2017)

If the acquired data does not meet the above quality thresholds, then the test data is rejected and an error is displayed on the device with guidance of the corrective action. This is essential as it prevents reporting unreliable nerve conduction data from the NC-Stat device (Neurometrix Inc., 2017).

Lee et al. (2014) & Shibata et al. (2019), assessed 100 individuals living with diabetes mellitus to evaluate the intra- and inter- examiner reliability of the DPNCheck. The assessment of the diabetic population is important and this is a challenging cohort for any physiological test given the age, elevated BMI and many comorbidities in this population. DPNcheck exhibited good to excellent reliability when individuals with diabetes were investigated as well as achieving exceptional validity. The intra- and inter-examiner reliability was compared and it was demonstrated that the variability had no impact on the diagnostic accuracy.

The below table (table 5) summarizes the result of seven published studies in high quality peer-reviewed journals that evaluated the diagnostic accuracy of DPNCheck for the detection of DPN by investigating its sensitivity and specificity. In total, all of the studies mentioned below assessed around 900 participants with either type 1 or type 2 Diabetes Mellitus as well as non-diabetic controls. The reference standard for the diagnosis of peripheral neuropathy was either established by comprehensive clinical tests such as the neuropathy disability score (NDS) or the Toronto clinical neuropathy score (TCNS) or traditional NCS as performed or supervised by a neurologist. The DPNCheck exhibited high diagnostic sensitivity into detecting peripheral neuropathy, as about 9 out of 10 patients were identified with acceptable rates of false positives. It is important to point out that the reference diagnosis for peripheral neuropathy, against which DPNCheck was compared were themselves not perfect particularly the clinical methods and therefore the actual performance of DPNCheck is likely better than that represented here (Gozani, 2020).

Table 5- Sensitivity and Specificity of the NC-Stat® DPN Check®

Study Publication	Type 2	Type 1	No Diabetes	Total	Reference Diagnosis	Sensitivity	Specificity
Binns-Hall et al. 2018	231	5	0	236	Clinical	0.84	0.68
Papanas et al. 2019	0	53	0	53	Clinical	0.96	0.93
Chatzikosma et al. 2016	114	0	46	160	Clinical	0.91	0.86
Hirayasu et al. 2018	92	0	0	92	Clinical	0.85	0.86
Lee et al. 2014	28	16	0	44	NCS	0.95	0.71
Kural et al. 2018	168	0	0	168	NCS	0.82	0.85
Scarr et al. 2018	0	68	71	139	NCS	0.86	0.79
Total	**633**	**142**	**117**	**892**		**0.88***	**0.82***

Summary sensitivity and specificity determined by bivariate meta-analysis.

Youden Index = 0.70 (effective diagnostic test has Youden Index > 0.50, Power et al. 2013)

(Gozani, 2020)

As per table above, the DPNCheck obtained a Youden index of 0.7 which is considered highly effective particularly for a physiological test as a benchmark comparison. The Youden index for detecting diabetes by HBA1C is around 0.5 and for prediabetes it is around 0.25. Similiarly the Youden Index for detecting peripheral arterial disease by Ankle-brachial pressure index (ABPI) is around 0.5. In fact the DPNCheck diagnostic performance is equivalent to the comparison of diagnostic assessment of neuropathies by two neurology laboratories. This indicates that in the clinic one can have a high degree confidence that the DPNCheck is correctly identifying the peripheral neuropathy status of the patient (Gozani, 2020).

Moreover, a study conducted by Chatzikosma, et al. (2016), showed that the positive predictive value of the NC-Stat tool was found to be 79.17% whilst the negative predictive value was slightly higher that of 93.94%. Also, this study obtained a positive likelihood ratio of 6.51 and a negative likelihood ration of 0.11.

It was also reported that an elevated HBA1C was found to be related to the large nerve fiber neuropathy complications which decreases motor and sensory nerve conduction velocities. This further highlights the importance of utilizing nerve conduction testing such as the NC-Stat® DPN Check® for early and accurate diagnosis of peripheral neuropathy in a diabetic population especially in the presence of longstanding uncontrolled hyperglyacaemia (Smith & Singleton, 2013).

The NC-Stat® DPN Check® has been shown to be a reliable diagnostic test, however it is still not considered as a ‘gold standard’ tool. This device assesses the large myelinated nerve fibers and will not identify any abnormalities of the small fibers. The large myelinated nerve fibers are responsible in mediating proprioception, vibration sensation and light touch. In DPN, it usually involves both large and small nerve fiber damage where some researchers state that small nerve fibers are affected earlier in DPN than large nerve fibers (Carmichael et al.,2021).

There are other factors that can affect nerve conduction testing which include excessive tissue such as oedema or adipose tissue, skin temperature, anatomical variation of the sural nerve, improper skin preparation and device misplacement. These factors can result in measurements that cannot be recorded, indicated by SNAP measuring less than 1.5μV (Lee et al.,2014).

Furthermore, the sensitivity and specificity of the results obtained can be slightly affected by the patient’s age and height. The test will show a maximal specificity in patients who are younger and shorter in height. Whilst on the other hand, older and taller individuals will indicate maximal sensitivity. Therefore, clinician should take into consideration any of these factors when interpreting results (Lee et al.,2014); (Shah et al.,2018); (Carmichael et al.,2021).

Another limitation of the device is the variation of SNAP and SNCV from test to test. This variability may be due to factors such as differences in device placement or nearby electrical interference. Neurometrix Inc. considers a variation of less than 5% for SNCV and less than 25% for SNAP as acceptable limits. In clinical practice, if a borderline result is obtained, it is recommended to repeat the test (Lee et al., 2014); (Pafili et al.,2017). Furthermore, the NC-Stat may not be readily available in primary clinics or used for routine screening due to it being more costly than other screening tools (Poulose et al.,2015).

The NC-Stat® DPN Check® has been proven to be an optimal modality utilized in confirming the diagnosis of DPN. However it has also been suggested that the diagnosis of DPN should not be solely based on this device but clinicians should also consider the medical history, physical examination and other objective test results (Carmichael et al.,2021).

2.12 Current Literature on Screening for Diabetic Peripheral Neuropathy

Numerous studies have investigated which screening method is optimal in diagnosing the risk of developing DPN. Since many individuals with DPN are initially asymptomatic, the importance of early assessment and the use of screening modalities to detect this progressive disease have been emphasized (Pop-Busui, et al., 2017).

From the available literature, it is evident that there is a significant variation in the reported prevalence of DPN across different studies. This variation can be attributed to several factors, including lack of standardized diagnostic criteria variations in the gold standard test utilized for diagnosis, differences in population awareness and other confounding factors such as the onset of diabetes (Ramanathan et al.,2021).

The lack of consistent diagnostic criteria is one of the primary reasons for the variability in prevalence rates. For instance, some studies have used VPT measured with biothesiometry as the gold standard, while others have relied on NCS. Even within NCS, there is no universally accepted criterion for diagnosis. Similarly, studies using VPT have employed different cutoff values such as 15V or exceeding 25V, to define peripheral neuropathy. These variations in diagnostic criteria may result in the misdiagnosis of subclinical cases of neuropathy (Liu et al.,2021); (Ramanathan et al.,2021).

Despite the controversies surrounding diagnostic criteria, many studies have considered NCS as the gold standard due to its objective and reliable nature. However, NCS is a time-consuming and technically challenging procedure. Therefore, it is important to explore alternative screening tools that can be used in a clinical setting (Lee et al.,2014).

This study aimed to compare various subjective screening modalities used for the detection of peripheral neuropathy in a clinical setting and compare each test to the reference tool namely NC-Stat® DPN Check® which is known for its reliability in objectively detecting neuropathy.

The NC-Stat® DPN Check® was compared to the Laser Doppler Imaging (LDI) Flare technique, a well-established method of early assessment of small nerve fiber dysfunction. This diagnostic non-invasive tool measures the neurovascular function and small nerve fiber dysfunction by assessing the blood flow and microcirculation in the skin. LDIFlare can detect early changes in peripheral neuropathy by measuring the increase in skin blood flow in response to local heating stimulus (Sharma et al.,2015).

The study included 80 health controls and 162 individuals with type 1 and 2 diabetes mellitus of ages less than 65yrs and a diabetic onset of more than 10 years. Significant correlations were observed between the NC-Stat tool and the LDI Flare in all stages of DPN. Notably there was a positive correlation even in subjects without clinical neuropathy suggesting that the device may be useful for assessing disease progression at early stages of neuropathy. However, the study highlighted that it is important to note that the NC-Stat device measures only large fiber function and does not assess small nerve fibers integrity. Nonetheless, this study provided evidence of significant linear relationships between the NC-Stat, LDI Flare technique and the clinical neuropathy scores. Thus, the findings suggest that the NC-Stat tool could serve as an additional diagnostic tool for diagnosing early DPN in a clinical setting (Sharma et al.,2015).

Another study by Shibata et al. (2019) compared the NC-Stat tool with the NCS namely a standard electromyography system (EMG) which is considered as a gold standard tool for detecting DPN. The study demonstrated a strong correlation between the two tests, indicating that the NC-Stat tool is a useful device for assessing DPN.

Furthermore, another study by Lee et al. (2014), compared the standard NCS with the NC-Stat device, which reported a high reliability and satisfactory accuracy. However, it was suggested to account for potential measurement bias, especially regarding SNCV and adjust threshold values to align with those of standard NCS.

Brown et al. (2017), reported that the use of the NC-Stat® DPN Check® is beneficial when incorporated with other low-cost screening modalities available in clinical settings for the early detection of peripheral diabetic neuropathy. This study also highlighted that the 128Hz tuning fork alone was not as accurate and therefore should be used as a tandem measure when screening. The research study had a limitation of having a small cohort of only 34 participants and may be insufficient in representing the larger population.

Another study investigated the Semmes-Weinstein 10g monofilament with the NC-Stat® DPN Check®. The results indicated that monofilament testing identifies later stage neuropathy and loss of protection sensation whilst sural nerve conduction testing detects an earlier stage of neuropathy. It was reported that 100% of the participants who obtained a positive monofilament test showed abnormal nerve conduction whilst 60% participants with a negative monofilament test were diagnosed with peripheral neuropathy due to abnormal nerve conduction (Pambianco et al., 2012).

The American Diabetes Association (2023) recommended that simple clinical modalities such as the Semmes-Weinstein 10g monofilament and the 128Hz tuning fork should be used as part of diabetic foot screening to identify the presence of peripheral neuropathy. Although, clinicians should keep in mind that that these modalities have limitations which include inter- and intra-analysis variability, lack of consensus regarding the assessment outcomes, subjective interference and inferior diagnostic sensitivity when compared with nerve conduction testing. It is suggested that the use of simple testing modalities alone is insufficient to diagnose the presence of DPN especially in its early stages. Therefore, the use of nerve conduction testing is recommended in confirming the diagnosis especially in individuals with a diabetic onset of more than 10 years (American Diabetes Association, 2023).

In a study conducted by Binns-hall et al. (2018), different screening tools including the Toronto Clinical Neuropathy Score (TCNS), 10-g monofilament, NC-Stat® DPN Check® and Sudoscan were compared for the early diagnosis of DPN and high- risk foot. The Sudoscan is a non-invasive test specifically developed to assess sweat gland secretory function as an indicator for peripheral neuropathy. The prevalence of DPN varied among the tools were the NC-Stat tool obtained the highest percentage of DPN at 51.5%, followed by Sudoscan at 38.2% then the TCNS at 30.9% and lastly the 10-g monofilament at 14.4%. This indicates that the NC-Stat device is more sensitive in detecting peripheral neuropathy especially when compared with the 10-g monofilament. Moreover, while the TCNS was considered the gold standard in this study, there is a lack of consensus regarding the true gold standard for assessing peripheral neuropathy.

In another study by Sheshah et al. (2020), the prevalence of DPN was compared using the neuropathy disability score (NDS) as the gold standard, along with the 10-g monofilament, NC-Stat® DPN Check® and Sudoscan. The prevalence rates varied between the tools where the highest percentage of limbs with DPN was obtained by the Sudoscan (73%), followed by the NC-Stat tool (40.9%), then the 10-g monofilament (19.5%) and lastly the NDS (13.8%). The study raised concerns about the possibility of overestimation of DPN prevalence by both the NC-Stat® DPN Check® and Sudoscan as well as the possible underestimation of the 10-g monofilament and the NDS. It is important to note that the designation of NDS as the gold standard is not universally agreed upon and the study did not account the duration of diabetes or age as contributing factors to neuropathy.

There is no current literature available that compares the neurothesiometer with the NC-Stat® DPN Check® for the diagnosis of DPN. The study carried out by Pafili, et al. (2020), was only intended to compare the effectiveness of the neurothesiometer and the NC-Stat tool among other screening tools for the diagnosis of cardiovascular disease.

There is also no literature comparing the 128-Hz ETF and the NC-Stat® DPN Check®. A study by Azzopardi et al. (2018), reported that from the three vibration screening modalities utilised in the study, the VibraTip showed to be more sensitive to vibration perception when compared with the other devices. The VibraTip device is similar to the ETF Constant mode as it generates a consistent vibratory stimulus and uses a similar method of application. The study indicated that 28.5% did not perceive vibration sensation when using the VibraTip whilst 21% and 12% were insensate to vibration when using the neurothesiometer and the 128Hz Tuning Fork respectively. It has been suggested that patients with diabetes should be examined with different modalities to assess neuropathy and when these do not concur further evaluation should be considered. This will lower the chance of falsely identifying patients as not having peripheral neuropathy (Azzopardi et al.,2018).

Moreover, various studies also suggest that employing multiple screening tools for the detection of peripheral neuropathy can significantly improve diagnostic accuracy and reliability (Hong, 2018) (Raymond et al.,2020).

Understanding that each tool has its advantages and limitations, the choice of screening tool may be dependent on factors such as cost, availability, expertise required and specific patient population being assessed. The lack of consensus on the ideal screening tool highlights the complexity of diagnosing peripheral neuropathy and the need for further research in this area. Future studies comparing the diagnostic accuracy, reliability and feasibility of different screening tools are necessary to establish evidence-based guidelines and recommendations for assessing peripheral neuropathy. Additionally, efforts to develop new technologies or refine existing tools may lead to more effective and reliable methods for screening and diagnosing peripheral neuropathy (Burgess et al.,2021); (Carmichael et al.,2021).

Currently, routine clinical practice lacks simple markers for the early detection of DPN. The measures used are limited and can only identify the disease at a late stage of its progression as deemed by various researchers. Even with standardized clinical assessments and scored evaluations, the interpretation remains subjective and heavily reliant on the clinician's judgement (Carmichael et al.,2021).

Therefore, the purpose of this study was to further investigate the effectiveness of subjective screening tools recommended in diabetic foot screening guidelines within primary healthcare settings for the detection of peripheral neuropathy and compare them to the objective nerve conduction testing using the NC-Stat® DPN Check®. The subjective screening tools chosen include the 10-g monofilament, 128-Hz TTF, neurothesiometer, and 128-Hz ETF and compared to the objective tool namely NC-Stat device which is not readily available in the local clinical setting due to its running cost.

This research study aims to shed more light on whether the current screening modalities for detecting neuropathy concur with each other in classifying limbs with peripheral neuropathy, when compared to NC-Stat testing. The NC-Stat device is considered an accurate and reliable quantitative screening tool for sural nerve conduction, known for its sensitivity and specificity in the early detection of DPN. For this reason the NC-Stat device was chosen in this study as an objective reference tool.

Chapter Three

Methodology

3.1 Research Design

This research study employed a prospective non-experimental quantitative study, using a comparative research design.

In a non-experimental study, the researcher relies on the observation and interpretation of data and the research study is usually descriptive or using a correlation research design. This means that the researcher is describing a relationship between two or more variables or describing a phenomenon as it stands with the use of graphs, percentages, averages and other statistical tests (Frey, 2018).

Moreover, a non-experimental research involves data collection from participants in their real-world or natural environment, without any interference from the researcher and without the need of including a control group (Chiang, 2015).

Furthermore, quantitative studies are descriptive, deductive and static. This type of research design emphasise on the validity of data and statistical measures where through the collection of numeric data it gives you the possibility to correlate two or more variables using statistical programs (Yilmaz, 2013)**.**

The prospective design involves selecting a specific population of interest and gathering the necessary information from a chosen sample. The design enables the researcher to carefully select participants based on specific criteria. Although, this process can be time-consuming in order to obtain a sufficient number of suitable participants (Nikolopoulou, 2022).

3.2 The Philosophical Aspect of the Study

The study adopted a post-positivist philosophical view, one which acknowledges that knowledge is developed through observations and measurements of the objective reality. Post-positivism challenges the notion of absolute truth in knowledge and recognizes that researchers studying human actions and behavior may not always achieve certainty in their claims of knowledge. Critical realists reject traditional positivist views and acknowledge the inherent imperfections and uncertainties in observations and measurements (Graffin, 2021).

To enhance the accuracy of understanding reality, post-positivism emphasizes the use of triangulation through multiple objective measures and adherence to standards of validity and reliability in quantitative research. In this thesis, the post-positivist philosophy was followed by utilizing previously developed and tested objective outcome measures (Lebow, 2021).

3.3 Validity and Reliability

The modalities chosen within a quantitative research should be proven to be reliable and valid. Both validity and reliability relate with replicability and stability when conducting tests (Heale & Twycross, 2015). Therefore, a test is deemed as reliable when the results obtained are reproducible when following the same method and are consistent over time. On the other hand, validity occurs when the results obtained accurately reflect what the device was intended to evaluate. This is measured by the sensitivity and specificity of the diagnostic tool (Heale & Twycross, 2015).

In this research study all the screening modalities/ methods chosen were proven to be valid and reliable by previous literature and recommended by diabetic foot screening guidelines which are essential when conducting a quantitative research.

3.4 Ethical Consideration and Permissions

The World Medical Association formulated the Deceleration of Helsinki to guide medical and health care professionals affiliated in clinical research about their duty to always safeguard the individuals participating in the study. Therefore, all investigations were carried out in accordance with the principles of the Declaration of Helsinki as revised in 2013, where it states that the researcher is to preserve dignity, life, health, autonomy, privacy and confidentiality to participants (Association World Medical, 2013).

Prior to commencing data collection, all the required permissions and approvals were sought. The research proposal, participants consent form and information sheet were sent and approved by the following cooperating institutions:

- Head of the Podiatry Department, Faculty of Health Science, University of Malta for permission to use vibrating tools available which include the neurothesiometer and NC-Stat DPN tool (Appendix 1).
- Professional Lead of Podiatry Services (Appendix 2) and the Primary Care Data Protection Officer (Appendix 3) to request permission to recruit individuals from Podiatry clinics at Primary Health Care Centres.
- Intermediary who is a state registered podiatrist working in a local Primary Health Care Centre for the recruitment of potential participant and handing out the information letter if voluntary interest is shown in taking part of this study (Appendix 4).

After all the above permissions were granted, approval from the Faculty Research Ethics Committee (FREC) and University of Malta Research Ethics Committee (UREC) were obtained (Appendix 5).

Before starting data collection, all participants were given both a verbal and written explanation in either English or Maltese (Appendix 6 & 7). The information letter included the purpose of this study, mode of assessment, the participant's involvement and their commitment should one show interest in taking part. Furthermore, participants were handed a consent form (Appendix 8 & 9) in their preferred language (English or Maltese) which highlighted the following points:

- Participation included non-invasive observations and examinations with no known or anticipated risks of harm.
- The data collected would be coded, securely stored anonymously and would not be identifiable when results are published in public domain.
- All information collected would be kept confidential and any personal information will be destroyed after completion of this study.
- This study followed the General Data Protection Regulation (GDPR) and national legislation.
- Individuals were voluntarily participating and could withdraw from this study at any given time without giving any explanation and would result in no negative repercussions.
- Participants would not receive any direct benefit for taking part in this study.

Any queries that the participants raised were clarified and were afterwards given time to make an informed decision should they wish to participate. Potential participants were then asked to sign the consent form if they decide to voluntarily take part in this research study. The consent form included the contact details of the researcher and supervisor respectively for any further questioning which could arise throughout the course of this study. This procedure of consenting to participate has been proven to be valid and with accordance to the Nuremberg Code (Ghooi, 2011).

3.5 Recruitment Method and Sampling

3.5.1 Sampling

The population targeted for this research included individuals with type 2 Diabetes Mellitus. The sampling frame selected were patients attending the Podiatry clinic in Primary Health Care Centres who fit the inclusion criteria of this study.

Selecting the correct sampling technique is important as it will ensure accuracy in the results obtained as well as anticipating that the sample size collected represents the chosen population. Non-probability sampling is when the researcher chooses a subjective method process to select samples from the population of interest. This type of sampling is effective and implemented in research as it is cost effective when compared with other sampling techniques as well as it is more time efficient in completing research. It is therefore important that the researcher identifies which non-probability sample is employed in their research (Etikan, 2016).

For the purpose of this study, the non-probability technique chosen to recruit participants was the convenience sampling method. This is a type of non-random sampling where individuals who fit the criteria are available, willing to respond and easily accessible to the researcher. Most researchers utilize convenience sampling, as although it is ideal to use the whole population, it is impossible to achieve as the number is finite therefore this type of sampling contracts the number of participants required (Etikan, 2016).

3.5.2 Recruitment Selection of Participants

The intermediary approached any potential participants by verbally briefing them on the nature of the study and whether they would be interested in taking part. The individuals who accepted to participate were further screened by the intermediary to verify that the participant fit the inclusion criteria. Furthermore, the intermediary handed an information sheet to the participant explaining in detail the procedures that would be carried out during data collection.

The screening by the intermediary consisted of asking questions such as medical history, social history, duration of diabetic onset, age, history of trauma, weight and height. Following this a vascular assessment was performed using spectral Doppler waveform analysis which is a non-invasive clinical test used to detect peripheral arterial disease. The use and result interpretation of the Doppler Ultrasound has been proven to be reliable and valid when used from an experienced podiatrist (Guilcher et al.,2021). The intermediary assessed both dorsalis pedis and posterior tibial artery and only participants with triphasic waveforms were recruited for this study to ensure that peripheral arterial disease was not present since patients with inadequate arterial perfusion are reported to cause microvascular effects of peripheral neuropathy. Individuals who fulfilled the inclusion criteria were eligible to participate (Young, et al., 2013).

3.6 Inclusion and Exclusion Criteria for Participation

Sixty-three individuals with type 2 diabetes mellitus who attend the Podiatry Clinic at Primary Health Care Centres were recruited through an intermediary. Participants were recruited on a “first through the door” basis which was considered the best method suited for this research study due to the time constraints for data collection (Etikan, 2016). Each individual participated voluntarily, and no inducement was offered.

The inclusion and exclusion criteria should be properly and clearly established by the researcher to ensure that there was no selection bias when it comes to recruitment as well as the results obtained to be deemed reliable (Popovic & Huecker, 2023).

Inclusion Criteria:

- Participants aged from 18 to 65 years (Hicks et al.,2021)
- Both males or females (Hamba et al.,2020).
- Adults diagnosed with type 2 Diabetes Mellitus as defined by the WHO (2020) and living with diabetes for at least 10 years (Ang et al., 2014).
- Participants with no peripheral arterial disease (Kim et al.,2014)

Exclusion Criteria:

- Participants living with Type 1 Diabetes Mellitus (Brown et al.,2017)
- History of neurological problems other than neuropathy such as nerve root compression and cerebral vascular disease (Kim et al.,2014)
- History of hypothyroidism (Gupta et al.,2016)
- Individuals with hepatitis B, Hepatitis C or HIV (Puplampu et al.,2019)
- Previously an alcoholic or diagnosed with alcoholic liver disease (Jones et al.,2020)
- Participants who were using recreational drugs or illegal substances (Jones et al.,2020)
- Participants with implanted electronic devices (Cronin et al.,2013)
- Any known history of damage to the lower extremities such as trauma which may have caused nerve damage (Brown et al.,2017)
- Any current ulceration, broken skin or wounds on the tested area (Brown et al.,2017)
- Participants who were pregnant at the time of the study (Huang et al.,2016)

- Degenerative disorders which affect the autonomic systems like Parkinson's disease, multiple system atrophy or Lewy-body disease (Palma & Kaufmann, 2018)
- History of cerebrovascular accident (CVA) (Brown et al., 2017)
- Severe oedema or adipose tissue (Brown et al., 2017)

3.7 Data Collection

3.7.1 Patient Characteristics

Sixty-three individuals who fitted the inclusion criteria were approached by the intermediary and voluntarily decided to participate in this study. Prior to data collection, the researcher explained in detail what the study entailed and the participant's involvement. Time was allocated for any queries or concerns the participant might have had and were clarified accordingly. Participants were required to sign the consent form for data collection to commence.

Data collection took place inside the Podiatry Clinic at a Primary Health Care Centre from where the participant was recruited. The whole process of assessment lasted approximately 1 hour to complete where both limbs were assessed for each participant. Data collection was carried out by the same investigator to ensure consistency in the data gathered.

The 5 different testing tools used in this research study include the 10g monofilament, 128-Hz TTF, 128-Hz ETF, neurothesiometer and the NC-Stat® DPN Check®. The Data Collection Sheet (Appendix 10) was used to log in the results obtained during examination of each testing modality. The researcher also recorded demographic data together with the medical and social history of the participant in the data collection sheet. The table below (table 6) gives a description of the variables measured in this study.

Table 6- Description of medical and social history measured in this study

Participant Code Number:	
Demographic Data	
Age	
Gender	
Medical & Social History	
Body Mass Index (kg/m^2)	Anthropometric Measurements of both body weight and height to calculate the BMI (Wiggermann, et al., 2019).
Duration of Diabetes Mellitus	The year participants were diagnosed with the onset of Type 2 Diabetes Mellitus. The duration must be more than 10 years of onset (Hamba et al.,2020).
Blood Glucose Level (mmol/L or mg/dL)	The latest value of fasting blood glucose concentration. For a normal result, the value should be between 70mg/dL (3.9 mmol/L) to 100mg/dL (5.6 mmol/L) (Felner & Umpierrez, 2014).
Glycated Hemoglobin (HbA1c)	The most recent value of HbA1c which is the average of blood glucose levels for the last two to three months. The optimal value for HbA1c level is 48 mmol/mol (6.5%) (Sherwani et al.,2016).
Smoking Status	If the participant was a smoker or had a history of smoking

	(Brown et al.,2017).
List of Medication	Participants were asked if they were on any medication such as Oral antihyperglycemic agents (OHAs), statins, anti-hypertensive or any other medication
History of Surgical procedures	If the participant had undergone any surgical intervention/s relevant to the lower limbs

Medical data required was retrieved from the hospital database namely I-Soft.

3.8 Procedure

Prior to the commencement of any assessments, it was ensured that the clinic temperature was between 25-30 °C. Maintaining a consistent room temperature is important as low temperatures may provide different readings due to its effect on nerve conduction (Chatzikosma et al.,2016).

All the examinations were carried out whilst the participant was static and non-weightbearing. Participants were asked to expose their limbs up to the knee and to lie in a supine on the clinical couch with their knee extended. Skin preparation was then carried out by the research by swabbing with 70% alcohol. Additionally, to maintain infection control, all tools were appropriately cleaned after each participant by swabbing with alcohol.

The following section described the methodological approach adopted in this study during data collection when utilizing the 5 different screening modalities for DPN.

3.8.1 Semmes-Weinstein 10-g Monofilament

The first screening modality used was the Semmes-Weinstein 10-g monofilament which is a subjective, user friendly, inexpensive tool commonly used by many clinicians to diagnose loss of protective sensation (Al-Muzaini & Baker, 2017).

Firstly, it was explained that only a light touch was to be felt when applying the monofilament to different areas on the feet. To assert that this was understood, the monofilament was applied to the participant's wrist to familiarize themselves with the expected sensation (Leese et al.,2011).

While the participant was asked to look away, the nylon monofilament was applied with sufficient force which caused the nylon filament to buckle (Fig.18). The total time contact time that the nylon filament was placed on the skin was approximately 2 seconds. The participant was then required to affirm every time that the nylon filament was felt (Spruce & Bowling, 2012).

Figure 18- Application of 10g Monofilament

(Potter, 2022)

Filament placement was avoided on callused or scarring sites as this is known to inhibit the sensation produced by the tool (Al-Muzaini & Baker, 2017).

For the purpose of this study, 10-point sites (Fig. 19) were tested in a random order on each foot as follows:

- plantar aspect of the first, third and fifth toe (Zhang et al.,2018)
- plantar aspect of the first, third and fifth metatarsal head (Zhang et al.,2018)
- plantar-medial aspect of the mid-foot (Zhang et al.,2018)
- plantar aspect of calcaneus (Zhang et al.,2018)
- dorsum-medial aspect of mid-foot (Zhang et al.,2018)
- dorsal aspect between the base of the first and second metatarsal (Zhang et al.,2018)

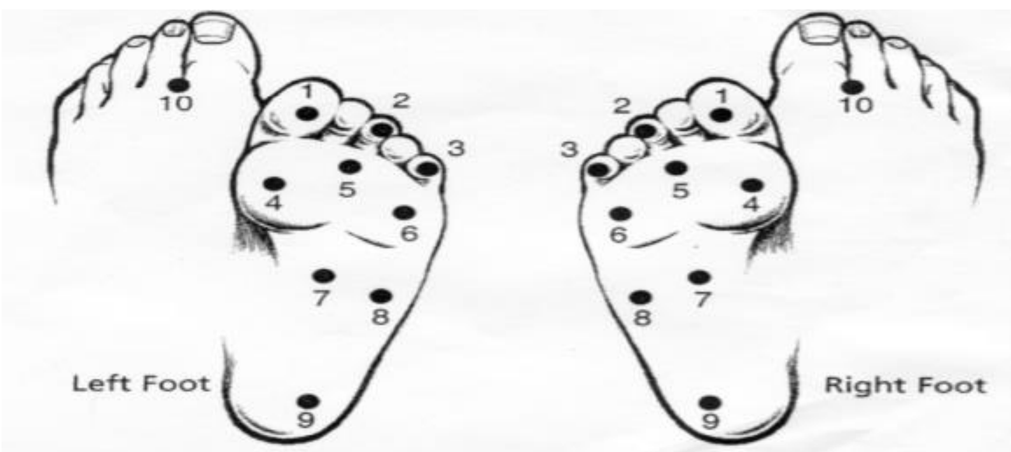


Figure 19- Diabetic Foot Screening 10- point test sites

(NHS, 2014)

This examination on each site was repeated three times. If the individual did not identify a point more than twice then that site was considered having loss of protective sensation. If all of the 10 sites were felt then participant was classified as having present sensation meaning ‘non-neuropathic’ whilst if at least one site was not felt then the foot is classified as having absent sensation meaning ‘neuropathic’ (Spruce & Bowling, 2012).

3.8.2 128Hz Tuning Fork

The 128Hz Tuning fork is widely used, cost-effective, readily available subjective screening tool used to identify the vibration perception as part of the diagnosis for DPN (Yang, et al., 2018). The timed assessment method relies on the principle that the amplitude of the stimulus diminishes gradually over time and considers the duration for which a patient can detect the vibration of the tuning fork. The outcome measure is determined by comparing the duration of vibration sensation felt by the patient with that of the clinician (Al-Muzaini & Baker, 2017); (Brown et al.,2017).

The technique used in this study included firstly striking the tuning fork against the researcher’s palm to initiate vibration without any audible humming to ensure that no excessive force was applied as it would affect the stimulus and increases the time of tuning fork vibration. If a massive clang was produced the researcher dampened the force and repeated the strike again with less force. Moreover, to ensure accuracy in the results obtained, the examiner was required to hold the tuning fork from the stem with two fingers and avoid making contact with the vibration tines as it decreases the vibration time produced (Barohn & Amato, 2013); (Al-Muzaini & Baker, 2017).

Figure 20- Application of 128Hz Tuning Fork

(Baker, 2005)

Prior to initiating the assessment, the vibration sensation is applied to the patient's hand to familiarize with the sensation. Once the vibration was initiated, participants were asked to avert eyes from the researcher and the base of the tuning fork was applied to the apex of the hallux (Fig. 20). The participants were then instructed to state the type of sensation felt and the exact moment the vibration perception ceased. Immediately after the participants no longer felt the vibration, the tuning fork was then placed on the dorsum bony prominence of the researcher's thumb and if vibration was still present, the time it took to completely cease was recorded (Al-Muzaini & Baker, 2017).

If the vibration persisted for at least 10-seconds after the participants ceased to feel it then they were categorized as 'abnormal'. Alternatively, participants who were unable to properly feel the vibration perception were considered as neuropathic (Al-Muzaini & Baker, 2017).

Therefore, for the purpose of this study, participants were categorized into two groups; either listed as 'present sensation' for individuals who felt the vibration stimulus or 'absent sensation' for those who did not satisfy the criteria (Al-Muzaini & Baker, 2017).

The procedure was applied bilateral on the apex of the hallux and repeated three times where an average was taken. Therefore, if the patient fails to correctly identify the vibration sensation on more than two occasions, it is considered as loss of vibration perception on that foot (Takahara et al.,2014).

3.8.3 Neurothesiometer

Another tool utilized in this research was the Neurothesiometer (Fig. 20) a user-friendly and portable tool that measures large fiber nerve function in a quantifiable manner from 0V to 50V (Richard et al.,2012).

The neurothesiometer consists of a stylus attached to a device that transmits a vibration stimulus at a given frequency controlled by the examiner. Prior to testing, the examiner familiarized the participant with what vibration that sensation was expected to be felt by applying the stylus to the distal palmar aspect of his/her hand. First, the participants were positioned supine and instructed to close their eyes to eliminate visual cues. The stylus was placed on the distal plantar aspect of the hallux (Fig. 21) with the voltage starting from 0 and gradually increasing at a rate of 1mV/s until the patient reports the initial sensation of vibration. At this point, the vibration intensity is recorded as the vibratory perception threshold. Three trials were performed on both feet where the mean voltage was calculated and recorded. The intensity and the change of intensity speed were under the researcher's control (Bracewell et al., 2012).

Figure 21- Neurothesiometer

(Edmonds & Sumpio, 2019)

The participants were classified as shown in the table below (Table 7) depending on the mean voltage obtained.

Table 7- Neurothesiometer Neuropathy Risk Classification

Voltage	**Risk Classification for Neuropathy**
0 to 20.99 V	Low risk
21 to 30.99V	Medium risk
≥ 31V	High risk

(Dubey et al.,2022)

3.8.4 128-Hz ETF

The O’Brien 128-Hz ETF is an easily operated and portable modality assessing the individual’s vibration perception threshold. It produces an almost silent vibration with constant amplitude and a frequency similar to that of a tuning fork. The ETF is composed of a contact tip from which the vibration stimulus was felt and the device’s body where the tool is activated and an integrated timer is initiated (O’Brien & Karem, 2014).

Both the descending mode and the constant mode were chosen for this study since both methods mimic the timed vibration tests and the on/off test of the TTF respectively. The tested area was prepared by swabbing with a 70% alcohol and the vibration stimulus was applied on the participant’s hand to familiarize with the sensation before initiating the test. Furthermore, the unit should be wiped clean with ispropyl alcohol and it was recommended that the contact tip was also wiped with alcohol before and after patient use for infection control purposes (O’Brien & Karem 2022).

Prior to initiating the test, the ETF was set to either descending mode or constant mode by selecting the appropriate option using the mode button on the device. This setting determined the specific method used for the assessment (O'Brien Medical, 2022).

Before starting the actual test, the participants were provided with a detailed explanation of what was expected from them during the assessment.

<u>128-Hz ETF Descending method</u>

To begin the assessment, the examiner carefully placed the rounded contact tip of the ETF on the dorsal aspect of the distal phalanx of the hallux, specifically on proximal to the nail bed, which served as the testing location (Fig. 22). The ETF should be held perpendicularly to the skin during testing. Any off axis positioning may result in less than optimal vibration transmission.

Once the ETF was properly positioned, the examiner initiated the test by pressing and holding the "run" button on the device. This action simultaneously activated the vibration and the built-in timer (O'Brien & Karem, 2014).

Figure 22- Application of the O'Brien 128Hz ETF

(O'Brien Medical, 2022)

During the test, the ETF generated a vibrational output that gradually decreased in amplitude from a high intensity to zero over a period of 25 seconds, mirroring the behavior of a TTF. The participants were experiencing the vibration sensation during this time and were informed to pay close attention to its presence. They were instructed to indicate the moment the vibratory sensation was no longer felt by saying "stop". This communication from the participant was important in determining the vibration disappearance threshold. The examiner promptly stopped the vibration and timer by releasing the "run" button on the ETF device (O'Brien & Karem, 2022).

Once the test was completed, the elapsed time in seconds, representing the duration of which the participant felt the vibration before it disappeared, was displayed on screen of the ETF device. This elapsed time was then compared to the reference scale provided on the device (Fig. 23) (O'Brien & Karem, 2014).

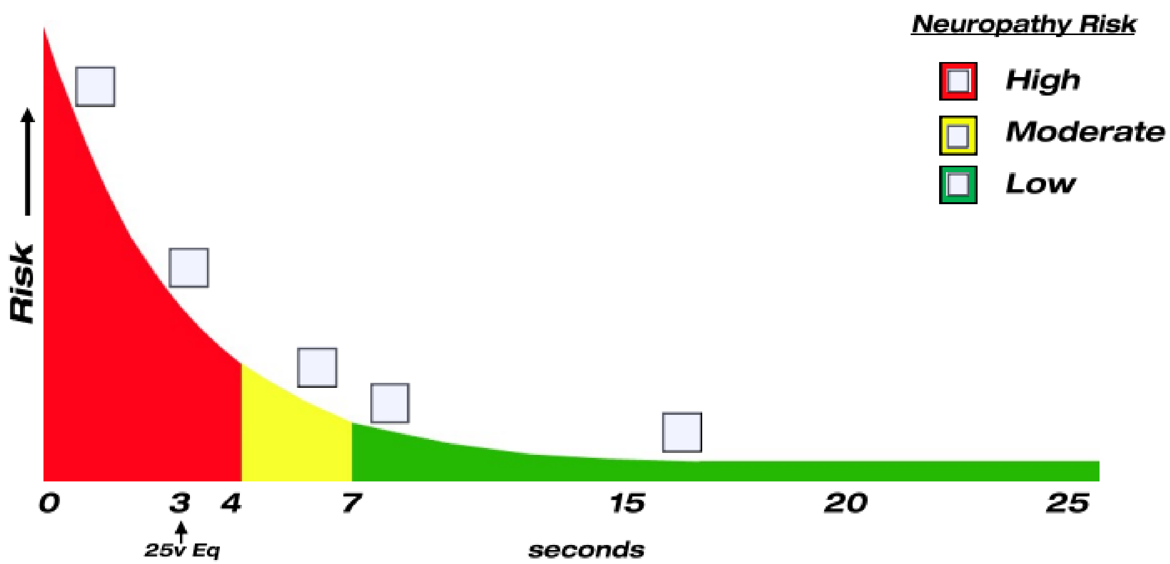


Figure 23- O'Brien 128-Hz ETF descending mode reference scale

(O'Brien Medical, 2022)

The reference scale helped assess the risk of neuropathy based on the elapsed time. As the built-in timer initiated and begins counting down from 25 seconds to 0, it is important to note that a longer duration suggests a decreased risk of neuropathy. Conversely, a shorter duration indicates a higher risk of neuropathy (O'Brien Medical, 2022).

Accordingly, the interpretation of results was as follows: a result ranging from 25 seconds to 7 seconds suggests a low risk of neuropathy. Results falling between 7 seconds and 4 seconds indicate a moderate risk of neuropathy. Finally, results below 4 seconds indicate a high risk of neuropathy. Moreover a result of 3 seconds is the equivalent of the 25V of the biothesiometer (O'Brien Medical, 2022).

The test was repeated for 3 times on each foot and a mean was obtained.

<u>128-Hz ETF Constant method</u>

The participants were asked to close their eyes during the duration of this examination. The examiner gently places the contact tip of the ETF device on the participant's hallux in the same anatomical area as that of the descending mode (Fig. 22). The contact was maintained for approximately a second and this process was repeated twice consecutively (O'Brien Medical, 2022).

In the first application, the ETF device was activated by pressing the "run" button and a vibratory stimulus was produced, while in the second application, the vibration was not present. The participant was then asked to differentiate between the two applications and determine in which application the vibration was felt. The procedure was repeated three times for both feet and the activation of the ETF device was randomized with each set of applications to prevent bias (O'Brien Medical, 2022).

If the participants were unable to differentiate between the two applications in more than two trials or did not feel any vibration stimulus, they were categorized as 'absent' sensation therefore neuropathic. On the other hand, if the participant successfully differentiates the presence or absence of vibration, they were classified as having ‘present’ sensation meaning non-neuropathic (O'Brien Medical, 2022).

3.8.5 NC-Stat® DPN Check®

The NC-Stat® DPN Check® is an accurate, non-invasive, fast and quantitative tool in assessing sural nerve conduction velocity and amplitude which are standard biomarkers for the diagnosis peripheral diabetic neuropathy (Poulose et al.,2015). It is user-friendly, portable and clinicians can interpret results easily. It is a handheld device consisting of two ankle stimulator rods, a sensory recording site where the disposable biosensor gets fitted and an infrared thermometer (Lee et al., 2014).

In this study, the NC-Stat device served as the reference tool against which other subjective tools were compared. This is because the sural nerve conduction device has been deemed by various studies as reliable tool for detecting peripheral neuropathy in its early stages as well as categorizing its severity (Shibata et al.,2019).

For the purpose of this study, the participants were asked to lay in a relaxed lateral recumbent position on the clinical couch with the leg to be tested on top and the other leg bent back towards the edge of the couch (Fig. 23). The researcher assessed that both participant’s lateral malleolus and Achilles tendon were visible. A preparation pad with 70% alcohol was used on the lateral malleolus and lateral lower 1/3 of the leg to swab the skin by removing any excess dry skin, lotions or oils (Brown et al.,2017).

Figure 24- Relaxed Lateral Recumbent Position for DPNCheck testing

(Neurometrix Inc., 2021)

The researcher proceeded to switch on the NC-Stat® DPN Check® tool after which the tail of the biosensor was then inserted into the connector port and attached to the adhesive foam to remain in place. Following this, the researcher was then required to set which leg was to be tested on the device. A small amount of conductive gel was applied on each of the two probes prior to applying the device on the skin. The short probe which is the Anode was aligned to the lateral malleolus bone and the long probe hence the cathode was placed adjacent to the central prominence of the lateral malleolus where the nerve was to be stimulated. The device was then aligned to the lower lateral calf and pushed down firmly by the researcher on the biosensor foam making sure that it is pointed towards the posterior knee with the inner edge of the biosensor placed next to the Achilles tendon. Following the correct positioning of the device (Fig. 25), the participant was informed to remain still and a sensation of around 10-12 non-painful mild pulses were delivered (Pafili et al., 2017). In the NC-Stat device utilized a built-in thermometer to address temperature fluctuations within the range of 23°C to 30°C. If the skin temperature fell below the acceptable range, the device alerted the operator, indicating that testing could not proceed until the appropriate temperature conditions were met (Brown et al.,2017).

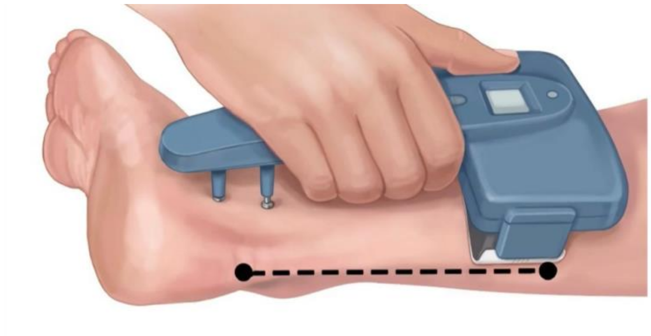


Figure 25- Positioning of DPNCheck Device on the lower leg

(Neurometrix Inc., 2021)

The researcher then proceeded to start the test where stimulation was delivered with every blink of light. A constant force was maintained for steady positioning throughout the duration of the test and results were displayed on the LCD screen after about 10-15 seconds (Pafili et al., 2017).

The results obtained were the sural nerve conduction velocity (meters/second- m/s) and amplitude (microvolts- μV) and were evaluated using the Sural nerve conduction guide. The sural nerve conduction velocity is usually between 20 to 70 m/s and a normal reading would be above 40m/s. On the other hand, sural response amplitude is usually between 0 to 32 μV and a normal reading is above 4μV. If the amplitude is 0μV it indicates that there is significant nerve fiber loss and severe neuropathy. Therefore, depending on the result obtained, participants were categorised as ‘normal’, ‘mild neuropathy’, ‘moderate neuropathy’ or ‘severe neuropathy’ on the data sheet according to the reference table guide as shown below (Fig. 26), (Sreenivasan et al., 2016).

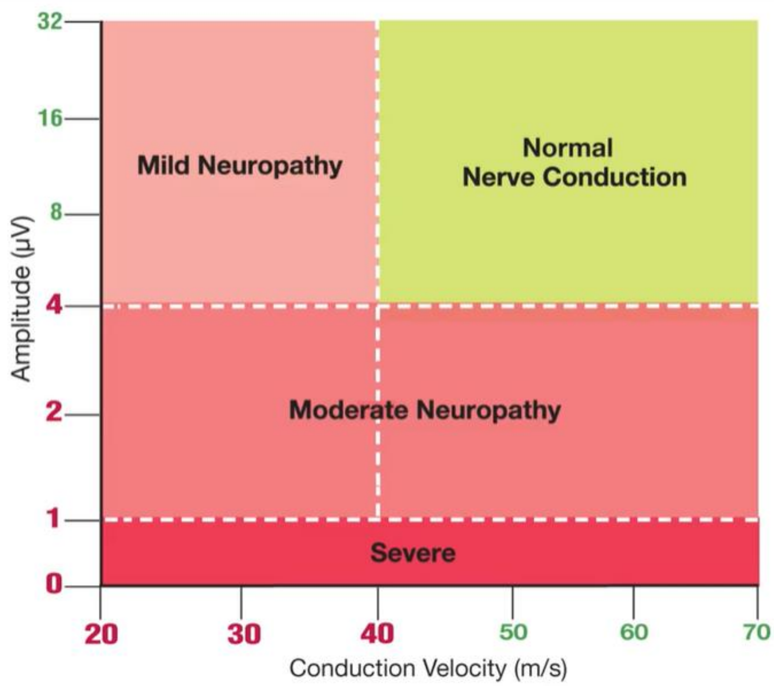


Figure 26- Reference Guide for Neuropathy Classification of DPN

(Neurometrix Inc., 2021)

The test was repeated when an error was displayed on the device which prevented from a reading to be recorded. However, only a maximum of five attempts were carried out as repeated stimulation of the sural nerve can potentially produce an error in the result obtained (Vinik et al.,2014); (Sharma et al.,2015).

Furthermore, if participants obtained an amplitude reading of 0, they were excluded from the study as it would not be possible to determine whether the individual is classified as having severe neuropathy or if it is due to the anatomical absence of the sural nerve (Lee et al.,2014).

Participants in the study were requested to attend only one session for data collection purposes. Detailed advice on general diabetic foot care, outlined in chapter 2.7, was provided to participants especially those who were identified as having signs of neuropathy risk from any of the screening tools mentioned above. The assessment of these tools was conducted by an experienced podiatrist, ensuring that proper evaluation and appropriate recommendations for foot care, based on the findings was provided.

3.9 Data Analysis

The data collected was recorded on a Microsoft Excel Spreadsheet and saved on a password-protected computer. All statistical tests were conducted using the IBM Corp SPSS (Statistical Package for the Social Sciences) software. Initially, prior to choosing the statistical tests, the One-Sample Kolmagrov-Smirnov test was used to assess whether data was normally distributed. Depending on the normalcy of data, parametric or non-parametric statistical tests were conducted. Moreover, descriptive and inferential statistics were used to quantitatively determine the main features of the data gathered and whether the particular sample represents the whole population.

The P value of less than 0.05 indicate that the alternative hypothesis. Statistical tests such as the Pearson, Spearman, Chi-squared, One-way ANOVA, multi-way ANOVA and contingency tables were used to assess the association between the values recorded. Further statistical analysis are discussed in Chapter 4 followed by a discussion in Chapter 5 where the results obtain in this research study were compared with the current literature.

Chapter Four

Statistical Analysis

4.1 Descriptive Analysis of Demographic Data

A total number of 63 participants (126 limbs) who satisfied the inclusion criteria took part in this research study. Out of the 63 participants, 39 (61.9%) were males whilst the remaining 24 participants (38.1%) were females. The mean age of participants was 59.16 years, were the youngest participant was 44 years and the eldest 65 years. The mean BMI for all participants was 30.62 kg/m^2 with the lowest BMI being 21.70 kg/m^2 and the highest BMI was that of 41.60 kg/m^2. The duration of diabetes of participants ranged between 10 to 38 years with a mean of 16.19 years. The mean HBA1C of participants was 7.89%, where the minimum HBA1C was 5.4% whilst the highest HBA1C was 14.30%, with a range of 8.90% (Table 8).

Table 8- Descriptive Analysis of the participating subjects (n=63)

Number of Participants	63
Male Participants	39
Female Participants	24
Mean Age (years)	59.16
Mean duration of diabetes (years)	16.19
Mean BMI (kg/m^2)	30.62
Mean HBA1C (%)	7.89

Participants who took part in this study were also asked about intake of medications (Table 9).

Table 9- Medications taken by recruited participants (n=63)

Medication	**Frequency**	**Percentage**
Oral hyperglycemic agents	63	100%
Insulin	14	22.22%
Statins	49	77.78%
Anti-Hypertensives	47	74.60%

Details regarding smoking habits (Table 10) were also gathered and presented below.

Table 10- Distribution of Smokers, Previous Smokers and Non-Smokers in the study group

	Frequency	**Percentage**
Current Smoker	10	15.87%
History of Smoking	24	38.10%
Non-Smoker	29	46.03%

4.2 Test for Normalcy

Prior to conducting statistical tests through SPSS, the continuous variables gathered during the study were tested for normalcy, as the choice for statistical test relies on the distribution of the data gathered.

The Kolmogorov Smirnov Goodness of Fit test (K-S Test) was used to determine if the continuous variables are normally distributed or not, as for sample size larger than 50 participants this test is more appropriate (Baghban et al.,2021). The result of the Kolmogorov Smirnov test would determine if parametric or non-parametric tests will be used to analyse further the data gathered during this study.

For the Kolmogorov Smirnov test, the following hypotheses were assumed:

Null Hypothesis : Data has normal distribution ($P>0.05$); (Dimitrina et al., 2020).

Alternative Hypothesis : Data has a non-normal distribution ($P<0.05$); (Dimitrina et al., 2020).

When the data gathered had a normal distribution, the parametric test One-Way Anova was chosen. Alternatively, if data gathered did not have a normal distribution, the non-parametric test Kruskal Wallis was used to analyse the data for any possible correlation (Xia, 2020).

The results of the Kolmogorov-Smimov test and any additional statistical analysis can be found in Appendix 11.

The continuous variables tested for normalcy in this study were the Neurothesiometer and the ETF 128-Hz descending. Both screening tools obtained a p-value of less than 0.05 indicating that the variables are not normally distributed, therefore the Kruskal Wallis had to be used when analysing continuous data.

4.3 Outcome Measures

The Chi-squared Test for independence (also known as the Pearson's chi- square test) is utilized to analyze if there is any association between two or more categorical variables in the data set provided (Turney, 2022). This test can only analyse the associations between categorical variables and does not indicate the cause of such inferences. Furthermore, this test is unable to compare and assess any relationship between categorical and continuous variables or between continuous variables.

To analyze the data provided, this test uses the contingency table (also known as the cross-tabulation) which arranges the data by consequently classifying it to the two categorical variables (Yeager & O'Neill, 2023).

In this study, the Chi-squared was used to determine any possible relationship between the following two variables:

1. The presences or absence of sensation from all the screening modalities
2. The commonly used screening modalities which included the Semmes-Weinstein 10g monofilament, 128-Hz Traditional tuning fork, Neurothesiometer, 128-Hz Electronic Tuning fork when compared to the NC-Stat® DPN Check®.

A p-value less than the level of significance ($P < 0.05$), is considered to be statistically different and therefore the null hypothesis is rejected whilst alternative hypothesis is accepted. Hence this shows that a significant difference between the two variables was found.

On the other hand, if the p-value is greater than the significance level ($P > 0.05$), the null hypothesis is accepted indicating that there is no significant difference between the column percentages between the two categorical variables, and it can therefore be concluded that both variables are not significantly different.

The Cramer's V is an extension of the chi-squared test of independence and it is used to measure the strength of association (effect size) between the two categorical variables. The result of this test has a metric scale which ranges from 0 to +1, where 0 indicating no association and +1 indicating strong association between the variables tested (Syed & Khan, 2020).

If the effect size value (Table 11) is less than 0.3, then the association between the variables is considered as weak even though the result is statistically significant. If the result is between 0.3-0.5 then the magnitude of effect size is moderate; whilst a value greater than 0.5 indicates that there is a strong association between the variables tested (Kim, 2023).

Table 11- Cramer's V Interpretation of Effect size

Effect Size (ES)	**Interpretation**
$ES < 0.3$	The result is weak. Although the result is statistically significant, the fields are only weakly associated.
$0.3 < ES < 0.5$	The result is moderate. The fields are moderately associated.
$ES > 0.5$	The result is strong. The fields are strongly associated.

(Cohen, 1988)

While the Cramer's V has its merits, there are also a few disadvantages which are important to consider. The Cramer's V is influenced by the number of categories within each variable. When variables have a large number of categories, Cramer's V tends to be inflated, potentially exaggerating the strength of association. Conversely, when variables have few categories, Cramer's V may under estimate the association. Furthermore, it does not take into account the distribution of the variables being analysed. As it only focuses on the association between categories and treats all categories as equally important. Consequently, it may not capture the nuances of the relationship, particularly if there are differences in the distributions of the variables (McHugh, 2013).

Furthermore, the Kruskal-Wallis Test is a non-parametric hypothesis test used when the continuous dependent variable is not normally distributed. This test investigates any statistically significant relationship between an independent group and a continuous dependent variable. As this test is non-parametric, it does not utilize the differences of the data but it alternatively conducts rank variance analysis (Lund & Lund, 2020).

If the p-value produced from this test is less than 0.05 level of significance, then the alternative hypothesis is accepted indicating meaning that there is a significant difference between the percentages obtained from each screening tool. On the other hand, if the p-value is more than 0.05 the null hypothesis is accepted therefore indicating no significant difference between the groups tested and that the percentages obtained are comparable (Hazra & Gogtay, 2016).

4.4 Distribution of Neuropathic risk for all Screening Modalities

The table below illustrates the results obtained from all the screening tools used in this research study. The screening modalities used in this study had different ways in categorizing the presence of neuropathy. Therefore, for the purpose of comparing all screening tools together, those with more than two categories were grouped as either having present sensation (in cases where the result suggested is normal or low risk of neuropathy), or absent sensation (for any other classification which ranges from mild to high risk neuropathy). This was implemented in order to statistically analyse and compare the results from all screening modalities used in this study.

The table below (Table 12) indicates the percentage of limbs which were classified as either have present or absent sensation for each screening tool (n= 126 limbs).

Table 12- The Percentages of limbs with no DPN and with DPN for each screening tool; p-value for chi-square test and Cramer's V test result

Neuropathy test * NeuropathicRisk Crosstabulation

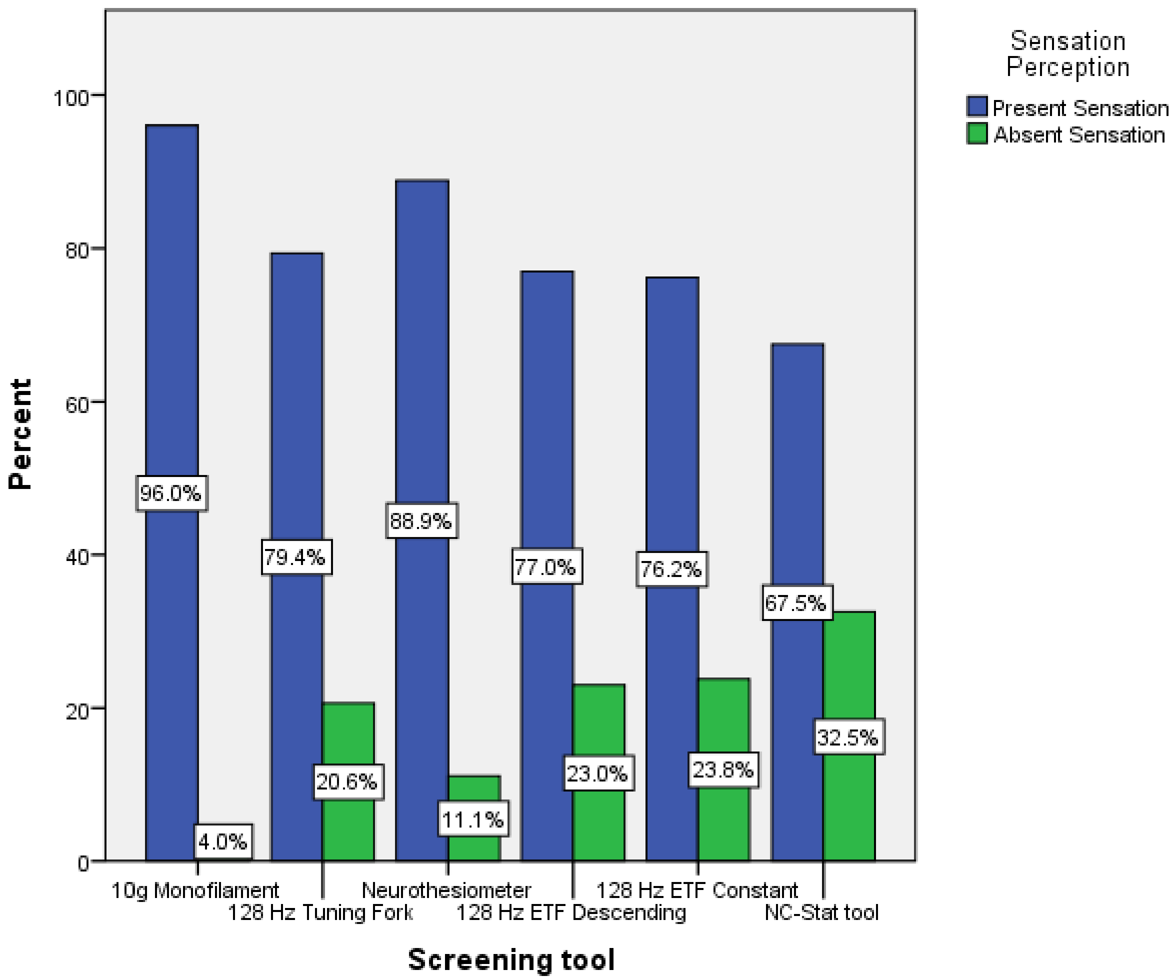
			Neuropathic Risk		
			Present Sensation	Absent Sensation	Total
Neuropathy test	10g Monofilament	Count	121	5	126
		Percentage	96.0%	4.0%	100.0%
	128 Hz Tuning Fork	Count	100	26	126
		Percentage	79.4%	20.6%	100.0%
	Neurothesiometer	Count	112	14	126
		Percentage	88.9%	11.1%	100.0%
	128 Hz ETF Descending	Count	97	29	126
		Percentage	77.0%	23.0%	100.0%
	128 Hz ETF Constant	Count	96	30	126
		Percentage	76.2%	23.8%	100.0%
	NC-Stat tool	Count	85	41	126
		Percentage	67.5%	32.5%	100.0%
Total		Count	611	145	756
		Percentage	80.8%	19.2%	100.0%

$\chi^2(5)$ = 41.719, p-value <0.005; Cramer`s V = 0.235

The Chi-Squared test was used to evaluate and assess any association between all the screening modalities chosen for this study. The p-value produced through the chi-squared test was 0.000 which is less than the 0.05 level of significance. Therefore, since the p-value is less than 0.05, the alternative hypothesis is accepted, meaning that the percentages of absent and present sensation varied significantly between screening tools. Moreover the Cramer's V value was that of 0.235 suggests that although a statistically significant difference exists between the percentages obtained, the practical or substantive significance of this difference may be small.

From the table above one can notice that the highest percentage of limbs which were classified as having absent sensation was through the NC-Stat® DPN Check® (32.5%), whilst the 10g monofilament had the least percentage of limbs which were classified as having absent sensation (4%). The 128 Hz ETF Constant and the 128 Hz ETF Descending had similar results, were 23.8% and 23% of limbs were classified as having absent sensation respectively. On the other hand 20.6% of limbs which were assessed through the 128Hz Tuning fork for neuropathy risk had absent sensation. Following, the Neurothesiometer has shown that 11.1% of limbs had absent sensation, where the vibration sensation was felt after the 21V.

The above percentages are better depicted in the cluster bar graph below (graph 1), where the number of limbs with or without the risk of peripheral neuropathy utilizing various screening tools frequently used was compared. This graph illustrates and highlights the variation in discrepancy between the results obtained from each of the screening tools used.



<u>Graph 1- Percentage of limbs with present and absent sensation for each neuropathy screening tool utilized in this study</u>

4.5 Each Screening Modality compared with Nerve Conduction tool

The aim of this research study was to compare the most frequently used screening tools in a clinical setting which give a subjective measure with the nerve conduction tool which gives an objective measure. In the following statistical analysis each tool has been individually compared with the NC-Stat® DPN Check® tool.

4.5.1 Relationship between the Semmes-Weinstein 10-g monofilament with NC-Stat® DPN Check®

The chi squared test was used to compare results with regards to the risk of peripheral neuropathy between the 10g monofilament test and the NC Stat DPN check. For the purpose of this test the NC-Stat results were classified into normal, mild, moderate or high neuropathy, whilst the 10g monofilament test for neuropathy was classified as either present or absent sensation.

Table 13- The Percentages of limbs with present or absent sensation determined by the 10-g monofilament compared with the NC-Stat® DPN Check® neuropathy classification; p-value for chi-square test and Cramer's V test result

10g Monofilament * NCStat Categories Crosstabulation

			NCStat Categories			
			Normal	Mild	Moderate	Total
10g Monofilament	Present Sensation	Count	85	5	31	121
		Percentage	70.2%	4.1%	25.6%	100.0%
	Absent Sensation	Count	0	2	3	5
		Percentage	0.0%	40.0%	60.0%	100.0%
Total		Count	85	7	34	126
		Percentage	67.5%	5.6%	27.0%	100.0%

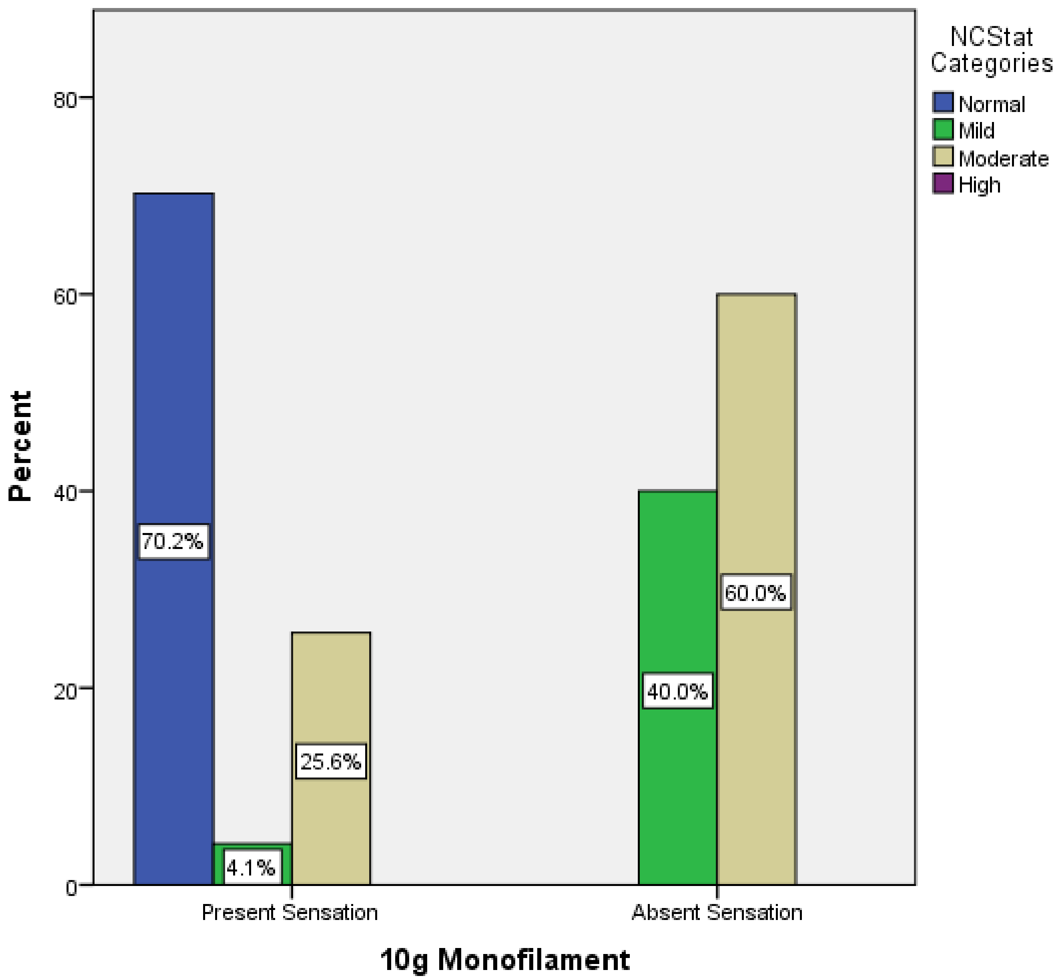
$\chi^2(5) = 16.735$, p-value <0.005; Cramer`s V = 0.364

Since the p-value for the Chi-square is less than 0.05 (P= 0.000), the null-hypothesis is rejected. This therefore indicates that the percentages obtained vary significantly between the 10-g monofilament and NC-Stat® DPN Check®.

The table above (Table 13) demonstrates that although 121 limbs were classified as having present sensation when assessed with the 10g monofilament, from these limbs 4.1% (5 limbs) had mild neuropathy and 25.6 % (31 limbs) had moderate neuropathy when classified through the NC-Stat tool. Therefore this shows that from the total percentage of limbs with present sensation as deemed by the 10g monofilament, only 70.2% of these limbs exhibited normal nerve conduction as determined by the NC-Stat tool. This can be better observed with the bar graph below (Graph 2).

Furthermore, there were only 5 limbs which were classified as having absent sensation when examined with the 10g monofilament, of which 40% (2 limbs) had mild neuropathy risk whilst the remaining 60% (3 limbs) had moderate neuropathy when classified according to the NC Stat DPNCheck.

Moreover, one can notice that from the data gathered there were no limbs which were classified as having high risk for neuropathy through the NC Stat DPN Check.



Graph 2- Percentage of limbs with present and absent sensation as per 10-g monofilament compared with the NC-Stat® DPN Check® neuropathy classification

Furthermore, when analyzing the strength of association between the two variables being tested through the Cramer`s V it was noted that there is a moderate effect size (0.364) between the 10g monofilament and the NC- Stat DPN check tool. This indicates that although there is a significant difference between the percentages obtained in the table above (table 13), a moderate effect size of Cramer's V suggests that the observed difference between the variables is of medium magnitude or strength.

However one should interpret the above results with caution since there is a large discrepancy in the number of limbs classified in each category for the 10g monofilament, where only 5 limbs out of 126 limbs which were classified as having absent sensation.

4.5.2 Relationship between the 128-Hz Traditional Tuning Fork with NC-Stat® DPN Check®

Since the data gathered for the 128Hz Traditional Tuning fork and the NC-Stat® DPN Check® are represented as categorical data, the Chi squared test was used to compare and assess for any association between the two screening tools for peripheral neuropathy. The results obtained from the NC-Stat are classified as either normal, mild, moderate or high neuropathy, whilst the 128Hz tuning fork test for neuropathy are classified as either present sensation or absent sensation.

Table 14- The Percentages of limbs with present or absent sensation determined by the 128- Hz traditional tuning fork versus the NC-Stat® DPN Check® neuropathy classification; p-value for chi-square test and Cramer's V test result

128-Hz Tuning Fork Hallux * NCStat Categories Crosstabulation

			NCStat Categories			Total
			Normal	Mild	Moderate	
128-Hz Tuning Fork Hallux	Present Sensation	Count	81	3	16	100
		Percentage	81.0%	3.0%	16.0%	100.0%
	Absent Sensation	Count	4	4	18	26
		Percentage	15.4%	15.4%	69.2%	100.0%
Total		Count	85	7	34	126
		Percentage	67.5%	5.6%	27.0%	100.0%

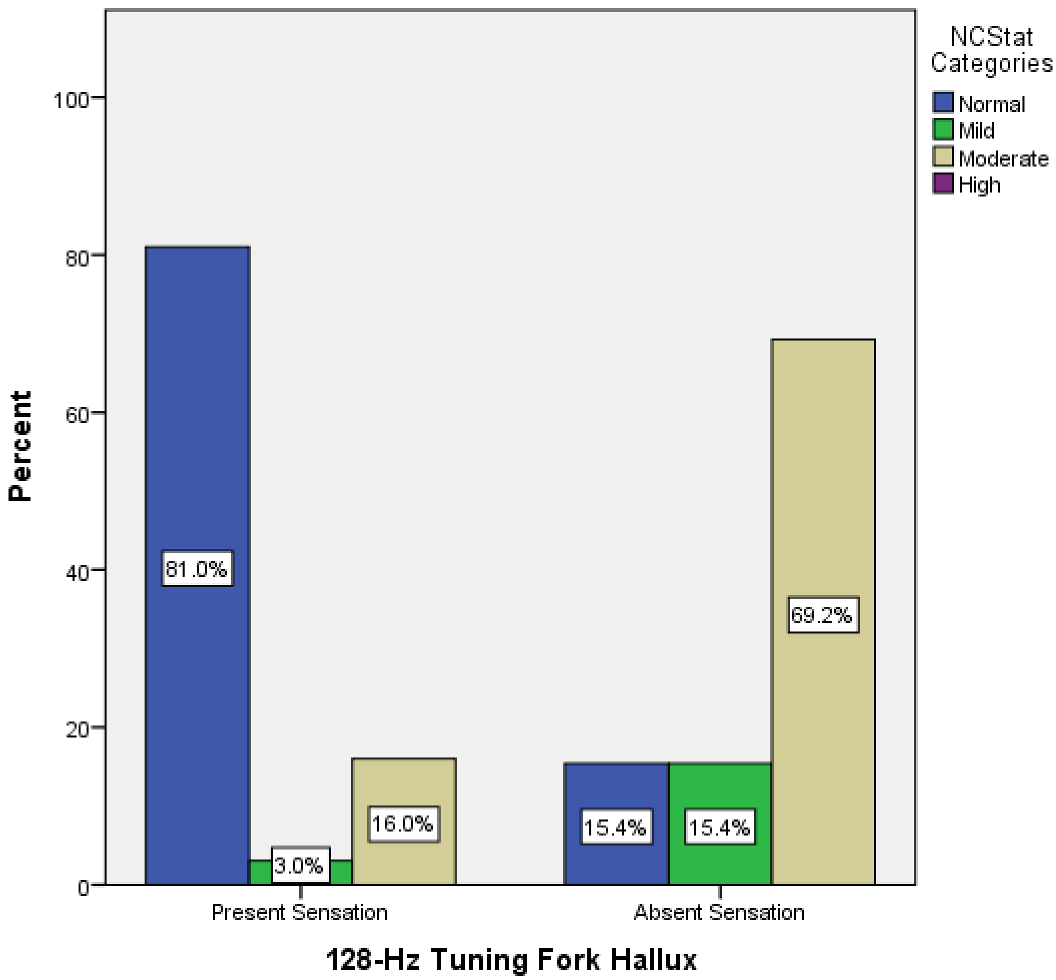
$\chi^2(5) = 40.534$, p-value <0.005; Cramer`s V = 0.567

The p-value obtained from the Chi-square was 0.000, which is less than the 0.05 level of significance. This means that the alternative hypothesis is accepted, and therefore indicating that there is a significant difference between the tools. Also, the Cramer`s V value of 0.567 suggests a strong effect size. Therefore, this means that the difference between the percentages obtained is of substantial magnitude or strength. Nonetheless, one should keep in mind the discrepancy between the present and absent sensation categories of the 128Hz tuning fork when interpreting these results.

From the above table (Table 14) one can observe that a total of 100 limbs were classified as having present sensation when using the 128Hz Tuning fork. However, the NC-Stat tool found that amongst those 100 limbs deemed as having present sensation by the tuning fork, 16% (16 limbs) were categorized as having moderate neuropathy whilst 3% (3 limbs) were classified as having mild neuropathy based on the NC-Stat results. Therefore, from the total amount of limbs classified as having present sensation with the tuning fork, 81% of those limbs also had normal nerve conduction with the NC-Stat tool since the remaining 19% had some form of peripheral neuropathy.

On the other hand 26 limbs were classified as having absent sensation utilizing the 128-Hz tuning fork test. From these limbs, 4 limbs (15.4%) were categorized as having normal nerve conduction with the NC-Stat® DPN Check® this possibly denotes a false positive result. So, among the limbs classified with absent sensation as per tuning fork, 15.4% exhibited mild neuropathy and 69.2% had moderate neuropathy when assessed using the NC-Stat tool.

The below cluster bar graph (graph 3) illustrates better the distribution of normal, mild, and moderate risk as classified through the NC-Stat® DPN Check®, in the absent and present sensation categories of the 128HZ tuning fork test.



Graph 3- Percentage of limbs with present and absent sensation as per 128-Hz traditional tuning fork compared with the NC-Stat® DPN Check® neuropathy classification

4.5.3 Relationship between the Neurothesiometer with NC-Stat® DPN Check®

The Kruskal Wallis was used to determine any association between the neurothethisiometer values and the NC-Stat® DPN Check® categories, namely normal, mild and moderate risk. The results obtained from the table below indicate that since the p-value obtained is less than 0.05, the alternative hypothesis is accepted (Table 15). In other words, this means that the results obtained from the neurothesiometer varies significantly in each category of the NC-Stat.

Table 15- Kruskal-Wallis P-value test between the Neurothesiometer and the NC-Stat® DPN Check® neuropathy classification

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Neurothesometer is the same across categories of NCStat Categories.	Independent-Samples Kruskal-Wallis Test	.001	Reject the null hypothesis.

Table 16- Descriptives from the KrusKal-Wallis Test between the neurothesiometer and the NC-Stat® DPN Check® neuropathy classification

	N	Mean	Median	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Normal	85	8.2761	8.0000	3.55598	0.38570	7.5091	9.0431
Mild	7	11.7857	11.0000	7.51015	2.83857	4.8400	18.7314
Moderate	34	13.8474	12.0000	7.26062	1.24519	11.3140	16.3807

Upon further evaluation, the above data (Table 16) one can notice that the mean value of the neurothesiometer results for the normal category of the NC-Stat® DPN Check® was that of 8.27V, whilst the median was 8V. The lower and upper bound of the 95% Confidence interval in the normal category of the NC-Stat were 7.51V and 9.04V respectively.

On the other hand the mean value of the neurothesiometer for the mild neuropathy category of the NC-Stat® DPN Check® was that of 11.8V, with a median value of 11V. The lower and upper bound of the 95% Confidence interval for the mild category of the NC-Stat were 4.84V and 18.73V respectively.

Lastly, the mean value of the neurothesiometer for the moderate category of the NC-Stat® DPN Check® was that of 13.85V, with a median value of 12V. The lower and upper bound of the 95% Confidence interval for the moderate category of the NC-Stat were 11.31V and 16.38V respectively.

The above results suggest that as the neuropathy classification of the NC-Stat® DPN Check® increases, the value gathered from the neurothesiometer neuropathy test generally increases as well. However, one should appreciate the fact that the number of limbs observed in each category of the NC-Stat varied significantly and therefore the results should be interpreted with caution.

Furthermore, from the pairwise comparison test as shown in the table below (table 17), one can appreciate that although the Kruskal wallis revealed a general discrepancy in the mean values obtained from the neurothesiometer when compared with the NC-Stat group categories (table 15); the true significant difference between these values was observed between the normal and moderate category of the NC-Stat.

Table 17- Pairwise Comparison between the neurothesiometer and the NC-Stat® DPN Check® neuropathy classification

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Normal-Mild	-18.498	14.356	-1.289	.198	.593
Normal-Moderate	-28.215	7.409	-3.808	.000	.000
Mild-Moderate	-9.716	15.153	-.641	.521	1.000

The Chi-squared test was also used to analyse any association between the neurothesiometer risk categories and the NC-Stat. For the purpose of this test the NC-Stat results were classified into either normal, mild, moderate or high neuropathy, whilst for the neurothesiometer results were classified as low risk (0-20.99 V), medium risk (21-30.99 V), and high risk (≥31 V) as per Dubey et al.,(2022).

From the below table (Table 18) and cluster bar graph one can notice that there were no limbs which were classified as high risk for the neurothesiometer neuropathy test.

Table 18- The Percentages of limbs with neuropathy risk category as determined by the neurothesiometer compared with the NC-Stat® DPN Check® neuropathy classification; p-value for chi-square test and Cramer's V test result

Neurotheisometer Category * NCStat Categories Crosstabulation

			NCStat Categories			
			Normal	Mild	Moderate	Total
Neurothesiometer Category	Low Risk	Count	84	6	22	112
		Percentage	75.0%	5.4%	19.6%	100.0%
	Medium Risk	Count	1	1	12	14
		Percentage	7.1%	7.1%	85.7%	100.0%
Total		Count	85	7	34	126
		Percentage	67.5%	5.6%	27.0%	100.0%

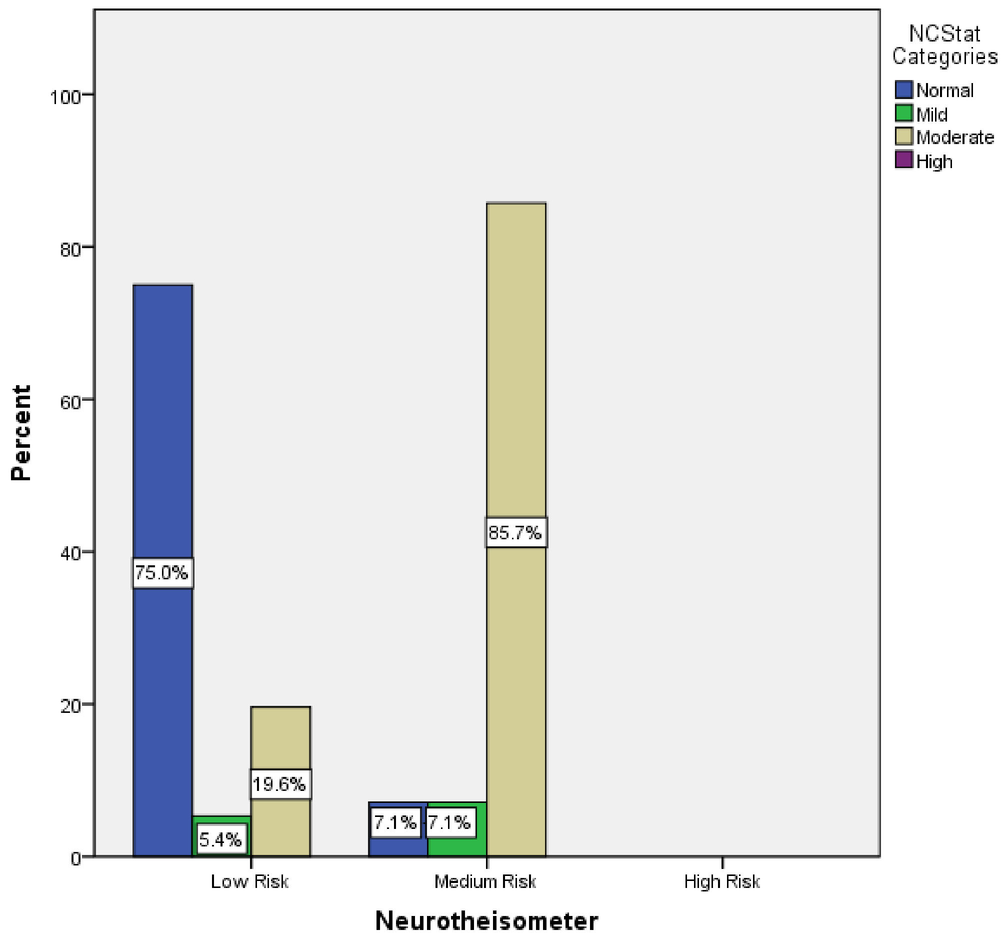
$\chi^2(5) = 28.698$, p-value <0.005; Cramer`s V = 0.477

The p-value of the Chi Squared test is less than 0.05 (P=0.000). This means that there is a significant difference between the results gathered from both screening tools. Furthermore, the Cramer`s V value obtained is that of 0.477, suggesting that the effect size between the two variables is of moderate strength.

The above table shows that a total of 112 limbs were classified as having low risk of neuropathy with the neurothesiometer. From these limbs, 84 limbs (75%) were classified as having normal nerve conduction with the NC-Stat tool. However, from the 112 limbs categorized as low risk by the neurothesiometer, 6 limbs (5.4%) were classified as having mild neuropathy and 22 limbs (19.6%) were deemed to have moderate neuropathy with the NC-Stat tool.

On the other hand, a total of 14 limbs were categorized as having medium risk of neuropathy with the neurothesiometer. From these limbs, 85.7% (12 limbs) were classified as having moderate neuropathy and 7.14% had mild neuropathy when assessed with the NC-Stat tool. However, 1 limb from the 14 limbs classified as medium risk with the neurothesiometer, was deemed as having normal nerve conduction with the NC-Stat tool.

The below cluster bar chart (Graph 4) gives on overview on the distribution of the NC-Stat results within the Neurothesiometer categories.



Graph 4- Percentage of limbs with neuropathy risk category by the neurothesiometer compared with the NC-Stat® DPN Check® neuropathy classification

4.5.4 Relationship between the 128-Hz ETF Descending with NC-Stat® DPN Check®

The Kruskal Wallis was used to compare the data gathered through the ETF descending variable with the NC-Stat since the data was not normally distributed. The p-value obtained from this test was that of 0.001 (Table 19). Since the p-value is less than the level of significance (0.05), the null hypothesis is rejected whilst the alternative hypothesis is accepted. Hence there is a significant difference between the observed variables.

Table 19- Kruskal-Wallis P-value test between the 128Hz ETF descending mode and the NC-Stat® DPN Check® neuropathy classification

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Average Descending is the same across categories of NCStat Categories.	Independent-Samples Kruskal-Wallis Test	.001	Reject the null hypothesis.

Furthermore, the pairwise comparison below has shown that the significant difference is mainly between the groups Mild to Normal and Moderate to Normal categories, where both had a p-value of less than 0.05 (table 20). This means that there is a statistical difference in the results obtained from categories highlighted in the table below (table 20) with the results of the NC-Stat device.

Table 20- Pairwise Comparison between the 128Hz ETF descending mode and the NC-Stat® DPN Check® neuropathy classification

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Mild-Moderate	-24.460	15.124	-1.617	.106	.317
Mild-Normal	43.369	14.328	3.027	.002	.007
Moderate-Normal	18.909	7.394	2.557	.011	.032

Table 21- Descriptives from the KrusKal-Wallis Test between the 128Hz ETF descending mode and the NC-Stat® DPN Check® neuropathy classification

	N	Mean	Median	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Normal	85	12.2612	11.5000	4.79559	.52015	11.2268	13.2956
Mild	7	6.3714	5.4000	2.79387	1.05598	3.7875	8.9553
Moderate	34	10.1824	9.5000	6.48450	1.11208	7.9198	12.4449

The above table (Table 21) gives a more detailed overview with regards to the distribution of the data gathered from the ETF Descending in the normal, mild and moderate categories of the NC-Stat.

One can appreciate that, the mean value of the ETF Descending results for the normal category of the NC-Stat® DPN Check® was that of 12.26 seconds, whilst the median was 11.5 seconds. The lower and upper bound of the 95% Confidence interval of the ETF Descending in the normal category of the NC-Stat were 11.23 seconds and 13.3 seconds respectively, whilst the standard deviation was that of 4.8 seconds.

On the other hand, the mean value of the ETF Descending for the mild category of the NC-Stat® DPN Check® was that of 6.37 seconds, with a median value of 5.4 seconds. The lower and upper bound of the 95% Confidence interval of the ETF Descending for the mild category of the NC-Stat were 3.8 seconds and 8.96 seconds respectively, whilst the standard deviation was that of 6.48 seconds.

Lastly, the mean value of the ETF Descending for the moderate category of the NC-Stat® DPN Check® was that of 10.18 seconds, with a median value of 9.5 seconds. The lower and upper bound of the 95% Confidence interval for the moderate category of the NC-Stat were 7.92 seconds and 12.44 seconds respectively, whilst the standard deviation was that of 6.48. However, one should interpret these results with caution since there is a large discrepancy between the number of cases in each group, where in the mild category there were only 7 limbs, whilst in the normal and moderate category were 85 and 34 limbs respectively.

Furthermore, the data gathered from the 128-Hz ETF Descending throughout data collection was categorized in a low, moderate and high risk for neuropathy as per O'Brien & Karem (2014). This data was then compared to the NC-Stat categories through Chi squared and the following results were obtained. The table below (table 22) demonstrates the distribution of limbs which were classified in different categories of neuropathy in both with the NC-Stat® DPN Check® and the ETF Descending neuropathy screening tool.

Table 22- The Percentages of limbs with neuropathy risk category as determined by the 128Hz ETF descending compared with the NC-Stat® DPN Check® neuropathy classification; p-value for chi-square test and Cramer's V test result

Category Descending * NCStat Categories Crosstabulation

			NCStat Categories			Total
			Normal	Mild	Moderate	
128-Hz ETF Descending	Low risk	Count	75	2	20	97
		Percentage	77.3%	2.1%	20.6%	100.0%
	Moderate risk	Count	7	3	9	19
		Percentage	36.8%	15.8%	47.4%	100.0%
	High risk	Count	3	2	5	10
		Percentage	30.0%	20.0%	50.0%	100.0%
Total		Count	85	7	34	126
		Percentage	67.5%	5.6%	27.0%	100.0%

$\chi^2(5) = 21.932$, p-value <0.005; Cramer`s V = 0.295

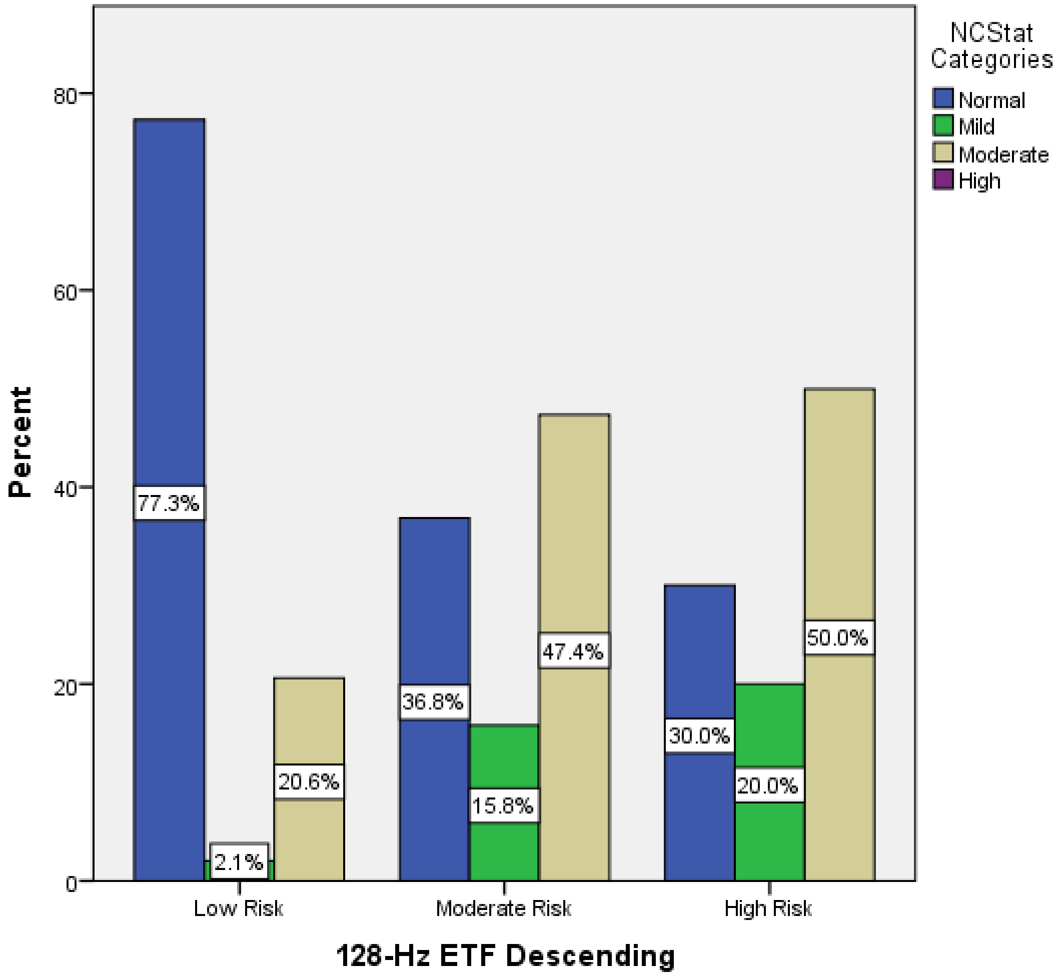
The p-value obtained is less than the 0.05 level of significance (P= 0.000) which indicates that the null hypothesis is rejected whilst the alternative hypothesis is accepted. Therefore, this shows that there is a significant difference between column percentages of the two variables tested namely the NC-Stat tool and ETF descending categories. These results were further evaluated through the Cramer`s V to determine the strength of this significant difference. The value of 0.295 suggests that the difference between the results obtained from each category is weak to moderate.

When observing the above table (table 22), it is demonstrated that a total of 97 limbs were classified as having low risk of neuropathy with the ETF descending mode. Out of these limbs, 75 limbs (77.3%) showed to have normal nerve conduction with the NC-Stat tool. However, from the 97 limbs which were deemed as low risk with the ETF, 20 limbs (20.6%) have moderate neuropathy and 2 limbs (2.1%) have mild neuropathy with the NC-Stat tool.

Moreover, 19 limbs were found to have a moderate risk of neuropathy in the ETF descending mode. From these limbs, 9 limbs (47.4%) have moderate neuropathy and 3 limbs (15.8%) have mild neuropathy when assessed with the NC-Stat tool. Furthermore, from the total of these 19 limbs categorized as moderate risk with the ETF, 7 limbs 36.8% were found to have normal nerve conduction by the NC-Stat tool.

Additionally, a total of 10 limbs, were classified in the high-risk category with the ETF descending mode. From these limbs, 5 limbs (50%) were classified as having moderate neuropathy and 2 limbs (20%) have mild neuropathy when assessed with the NC-Stat® DPN Check® However, out of these 10 limbs, 3 limbs (30%) were identified as having normal nerve conduction with the NC-Stat tool.

These assumptions have to be taken in the context that for each group, there was a large discrepancy in the amount of limbs observed in each category. The below cluster bar chart (Graph 5) gives on overview on the distribution of the NC-Stat results within the ETF descending mode category.



Graph 5- Percentage of limbs with neuropathy risk category by the 128Hz ETF descending mode compared with the NC-Stat® DPN Check® neuropathy classification

4.5.5 Relationship between the 128-Hz ETF Constant with NC-Stat® DPN Check®

The Chi-squared was used to compare and evaluate the association between the 128-Hz ETF Constant and the NC-Stat® DPN Check® as both tools provide categorical data. The ETF Constant were classified into two groups either present or absent sensation, whilst the NC-Stat were categorized as having normal, mild, moderate or high neuropathy.

Table 23- The percentages of limbs with present or absent sensation determined by the 128- Hz ETF constant mode compared with the NC-Stat® DPN Check® neuropathy classification; p-value for chi-square test and Cramer's V test result

ETF Constant * NCStat Categories Crosstabulation

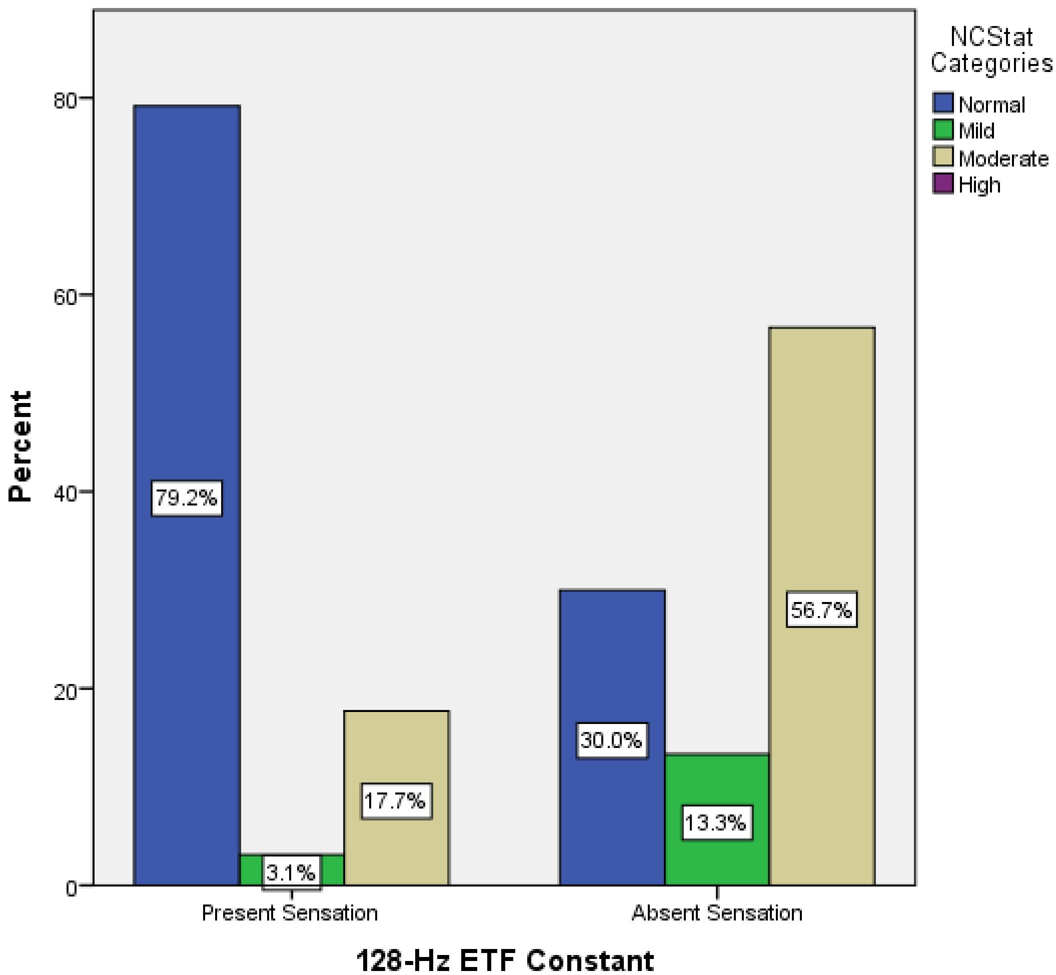
			NCStat Categories			
			Normal	Mild	Moderate	Total
ETF Constant	Present Sensation	Count	76	3	17	96
		% within ETF Constant	79.2%	3.1%	17.7%	100.0%
	Absent Sensation	Count	9	4	17	30
		% within ETF Constant	30.0%	13.3%	56.7%	100.0%
Total		Count	85	7	34	126
		% within ETF Constant	67.5%	5.6%	27.0%	100.0%

$\chi^2(5) = 25.334$, p-value <0.005; Cramer`s V = 0.448

The P-value produced by the Chi-square test as shown in the table above was less than 0.05 (P = 0.000), therefore the null hypothesis is rejected indicating that there is a significant difference between the two screening tools. Moreover, the Cramer's V value was that of 0.448 which shows that the discrepancy in the results obtained between the ETF constant and the NC-Stat® DPN Check® has a moderate effect size.

The above table (Table 23) shows that a total of 96 limbs were classified as having present sensation with the ETF constant. From these limbs, 76 limbs (79.2%) were also classified as having no neuropathy meaning normal nerve conduction with the NC-Stat® DPN Check®. However, 3 limbs (3.1%) have mild neuropathy and 17 limbs (17.7%) have moderate neuropathy as determined by the NC-Stat.

On the other hand, 30 limbs were classified as having absent sensation with the ETF constant. From these limbs, 17 limbs (56.7%) were identified as having moderate neuropathy and 4 limbs (13.3%) have mild neuropathy with the NC-Stat tool. However, among these limbs, a proportion of 9 limbs (30%) exhibited normal nerve conduction with the NC-Stat® DPN Check®.



Graph 6- Percentage of limbs with present and absent sensation as per 128-Hz ETF Constant compared with the NC-Stat® DPN Check® neuropathy classification

4.6 Summary of Results

A total number of 126 limbs were assessed utilized peripheral neuropathy screening tools as mentioned in the previous chapter. The following is a summary of the above results obtained in this research study:

- When comparing all screening tools for present/absent sensation, it was determined that there is a significant difference between the modalities utilized. The NC-Stat® DPN Check® had the highest percentage of limbs (32.5%) which were classified as having absent sensation. This was followed by the 128Hz-ETF constant (23.8%), 128Hz- ETF descending (23%), 128Hz TTF (20.6%) and Neurothesiometer (11.1%). The 10-g monofilament had the least percentage of limbs (4%) which were classified as having absent sensation. It can be noted that the percentage of absent sensation from the NC-Stat tool is significantly higher especially when compared with the neurothesiometer and the 10-g monofilament.

- A significant difference was observed between the Semmes Weinstein 10g monofilament and the NC-Stat® DPN Check®. This is because a percentage of limbs which were deemed as having present sensation with the 10-g monofilament actually exhibited mild or moderate neuropathy with the nerve conduction tool. Furthermore, despite 5 limbs being classified as having absent sensation with the 10-g monofilament were also found to have mild or moderate neuropathy with the NC-Stat, there was still a significant number of limbs did not correspond with the findings of the 10-g monofilament.

- The 128Hz Traditional Tuning fork demonstrated a significant difference when compared with the NC-Stat® DPN Check®. From the limbs classified as present sensation with the tuning fork, 81% of these limbs were also deemed to have normal conduction with the NC-Stat. However, it is important to note that a substantial percentage of these limbs despite being determined to have present sensation with the tuning fork, were classified as having mild or moderate neuropathy with the NC-Stat tool. Similarly in the absent sensation category of the tuning fork, while most libs with absent sensation also exhibited mild or moderate neuropathy as determined by the NC-Stat tool, there were still 15.4% of these limbs that showed nerve conduction. Hence, this suggests that there is a discrepancy between the two variables across all limbs assessed.

- The alternative hypothesis was accepted when comparing the neurothesiometer and the NC-Stat® DPN Check® indicating that there is a significant difference between the two variables. There were a significant amount of limbs which although were deemed as low risk with the neurothesiometer were found to have mild or moderate neuropathy with the NC-Stat tool. However, it was also observed that as the neuropathy classification of the NC-Stat® DPN Check® increases, there is a corresponding increase in the mean value obtained from the neurothesiometer neuropathy test, despite discrepancies in the results. Moreover, while there is a general discrepancy in the mean values between the neurothesiometer and the NC-Stat categories, the true significant difference was particularly evident between the normal and moderate category of the NC-Stat tool.

- The null hypothesis was rejected when comparing the 128Hz ETF Descending with the NC-Stat® DPN Check® meaning that there is a significant difference between the two variables. When assessing the data gathered through the pairwise comparison, it was determined that although there is a general discrepancy between the tools, the true significant difference was found between the mild to normal category and the moderate to normal category of the NC-Stat tool. Moreover, it was observed that while the majority of limbs categorized as low risk with the ETF descending were also classified as normal with the NC-Stat, there was a significant amount of limbs in the low-risk group that exhibited mild or moderate neuropathy according to the NC-Stat assessment. There were also limbs in the medium or high risk category of the ETF which were deemed as having normal conduction with the NC-Stat® DPN Check®.

- The alternative hypothesis was accepted when comparing the 128-Hz ETF constant and the NC-Stat® DPN Check® meaning that there was a significant difference between the two variables. Among the limbs classified as having present sensation with the ETF constant, a proportion of limbs also exhibited normal nerve conduction according to the NC-Stat tool. However, there was still a notable percentage of limbs in the present sensation category which showed to have mild or moderate neuropathy as assessed by the NC-Stat tool. Similar observation were made in the absent sensation category of the ETF, where 30% of limbs in this category showed normal nerve conduction as deemed by the NC-Stat tool.

- When interpreting the above results, one should take into consideration the notable discrepancy of limbs in each category of the screening modalities used. This implies that certain categories may have a limited number of limbs in comparison to other categories, where a substantial number of limbs were included.

4.7 Conclusion

In the following chapter, the above findings are discussed in further detail and compared comprehensively with the current literature available. Furthermore, the limitations and clinical implications of this study as well as the recommendations for future research were also addressed.

Chapter Five

Discussion

5.1 Introduction

This research study investigated and compared various screening modalities utilized within a primary care setting with the NC-Stat® DPN Check® for the detection of peripheral diabetic neuropathy. The NC-Stat device was used as an objective tool and compared with four subjective tools namely the Semmes Weinstein 10g monofilament, 128-Hz TTF, Neurothesiometer, O'Brien 128-Hz ETF. The screening tools chosen can all be used within a clinical setting. The examination and assessment was carried out following consent from participants with type 2 diabetes mellitus who met the inclusion criteria.

5.2 Modalities utilized for diagnosis of Diabetic Peripheral Neuropathy

5.2.1 Comparison between Screening Modalities

The study findings revealed a significant disparity in the detection of DPN when comparing the results from different screening tools. This discrepancy emphasizes the substantial variation in the effectiveness of screening modalities for identifying peripheral neuropathy in individuals with type 2 diabetes mellitus. It suggests that certain tools may possess higher sensitivity for detecting DPN when compared to others, further highlighting the need for careful consideration when selecting appropriate screening methods. This lack of agreement among the results obtained from all five screening tools indicates the potential occurrence of false positive or false negative outcomes for certain individuals.

Additionally, the variations observed among screening tools in this research study, further corroborates to the recommendations of other literature regarding the importance of using multiple modalities when screening for DPN (Azzopardi et al.,2018) (Hong, 2018)**;** (Raymond et al.,2020).

Among the screening tools used in this study, the NC-Stat® DPN Check® demonstrated the highest percentage (32.5%) of limbs indicating the presence of peripheral neuropathy. This implies that 32.5% of limbs were identified as having mild-to-moderate neuropathy, while 67.5% exhibited normal nerve conduction. Therefore, it can be inferred that among the available screening tools for clinical use, the NC-Stat device has the potential to be the most effective tool for the diagnosis of early stages of neuropathy. This finding aligns with previous research that compared the NC-Stat tool to gold standard tools in detecting peripheral neuropathy, further validating its efficacy. In fact, a study by Shibata, et al. (2017), demonstrated a good correlation between the standardized Nerve Conduction Studies (NCS) widely regarded as a gold standard tool for the early detection of neuropathy and the NC-Stat tool. Another study by Lee et al., (2014) also compared the standard NCS with the NC-Stat tool and reported an excellent reliability and acceptable accuracy.

Additionally, another study also demonstrated that the NC-Stat has a high level of sensitivity and specificity for detecting peripheral neuropathy at different stages and is well correlated with the Laser Doppler Imaging (LDI) Flare technique. The LDI Flare tool is capable of precisely measuring small fiber function and structure by assessing the blood flow and microcirculation in the skin. Small nerve fibers are affected earlier than larger fibers in peripheral diabetic neuropathy. Therefore, this demonstrates that the NC-Stat shows promising capabilities not only in the early diagnosis of neuropathy but also in monitoring the advancement of neuropathy in people living with diabetes (Sharma et al.,2015). Moreover, multiple studies have reported that the NC-Stat® DPN Check® the tool used in this study as an objective measure of DPN, showed high sensitivity and good specificity for the diagnosis of peripheral diabetic neuropathy in individuals with type 2 diabetes mellitus were percentages ranged from 84.3% to 95 % and 68.3 % to 86.11 % respectively (Chatzikosma et al.,2016); (Binns-hall et al., 2018); (Hirayasu et al., 2018); (Selvarajah et al., 2019); (Carmichael et al., 2021). So, based on the existing literature, the NC-Stat has been identified as a valid, reliable and sensitive screening tool for the detection of peripheral neuropathy.

The findings of this study may also suggest the possible implementation of the NC-Stat® DPN Check® as an objective measure for diabetic foot screening assessment for early detection of peripheral neuropathy.

Although, the NC-Stat device has shown to be effective in many studies (Lee et al.,2014); (Shibata et al.,2019) (Selvarajah et al., 2019) for diagnosing DPN in its early stages, it should be noted that this tool is not considered the gold standard test for the diagnosis of DPN so results should be interpreted with caution. A patient's medical history, physical examination and test findings should all be taken into consideration when making a diagnosis together with objective measures for DPN (Carmichael et al.,2021).

The O'Brien 128Hz Electronic Tuning Fork (ETF) reported the second and third highest percentage of limbs with risk for neuropathy. When the constant method was utilized 23.8%, of limbs tested were classified as having absent sensation, whilst when conducting the descending method 23% of limbs were categorized as having absent sensation indicating moderate to high risk of peripheral neuropathy. The O'Brien 128Hz produces vibration output at a decay rate similar to the traditional 128Hz tuning fork. Furthermore, the inclusion of an integrated timer in this tool facilitates the performance of accurate and reproducible timed vibration tests which have been demonstrated to be a valid method for detecting peripheral neuropathy (O'Brien & Karem, 2014). Unfortunately to date no literature is available in comparing the 128Hz ETF constant method with other screening modalities for the diagnosis of peripheral neuropathy.

In a study conducted by Azzopardi et al. (2017), a methodology similar to the current study was employed, with the VibraTip being used instead of the 128-Hz ETF constant method. In this approach, absent sensation was reported when the patient was unable to determine the presence of vibration between the two applications. The findings of this study revealed that when compared to the neurothesiometer and the 128-Hz traditional tuning fork, the VibraTip classified the highest number of limbs with absent sensation. Therefore, this suggests that the methodology used for both the Vibratip and the ETF 128Hz constant has demonstrated a high sensitivity in detecting peripheral neuropathy, since both tools outperformed other commonly used subjective screening tools. However Azzopardi et al. (2017), highlighted the possibility of false positive results since the results were not compared with nerve conduction testing.

Furthermore, available research is still limited when comparing the 128-Hz ETF descending with other screening modalities. The study conducted by O’Brien & Karem (2014), recommends the use of the ETF descending as the preferred tool for vibration testing in combination with Semmes-Weinstein monofilament testing for assessing lower extremity sensation in diabetic patients. The study compared the ETF descending method to the traditional 128Hz tuning fork and biothesiometer in terms of ease of use, testing time and standardization of vibration output. The authors concluded that the ETF was superior to the other devices and recommended its use.

However, the study is limited by a small sample size and the lack of comparison with a gold standard tool, which may have resulted in false-positive results. Moreover, further research is required to confirm the clinical utility of the ETF.

In this research study, the 128Hz ETF descending mode exhibited a higher percentage of limbs categorized as at risk of peripheral neuropathy than the other subjective screening tools assessed. This finding is consistent with the results of the study conducted by O'Brien & Karem (2014), which further supports the effectiveness of the 128Hz ETF as a screening tool for peripheral neuropathy.

Following the ETF, the 128Hz Traditional tuning fork reported that a total of 20.6% had absent sensation as they did not satisfy the criteria of perceiving vibration stimulus. A study conducted by Oyer et al. (2007), reported that the 128Hz tuning fork timed method detects the risk of neuropathy earlier than the 10g monofilament test. Moreover, Park & Kim (2019) found that the 128Hz tuning fork with timed method exhibited the second highest percentage, following ankle reflex of individuals with absent sensation in both asymptomatic and symptomatic in a type 2 diabetic population. Although, the study recommended the use of the 128Hz tuning fork for the clinical diagnosis of DPN, it also highly suggested using a combination of screening tools for a better outcome.

In another study, the effectiveness of the 128Hz tuning fork timed method was investigated by comparing its sensitivity and specificity to electromyography, where the results obtained were 21% and 88% respectively. However, this study evaluated not only the sural nerve but also the ulnar and median nerve for the diagnosis of neuropathy (Lai et al.,2014).

Despite the usefulness of the conventional tuning fork, some researchers question its effectiveness in reproducing results due to limitations related to both patient response and observer technique (Dubey et al.,2022). Another author suggested that the variation of results may be influenced by the vibration intensity, which is dependent on the striking strength of the tuning fork (Mclllhatton et al.,2021). Whilst another researcher argued that the lack of standardization and quantification of clinical outcomes may also contribute to the variability of results obtained by the tuning fork (O'Brien & Karem, 2014). Therefore, although some literature suggests that the 128Hz tuning fork is a sensitive indicator for peripheral neuropathy; other researchers question its diagnostic effectiveness. So, while the tuning fork was able to detect the presence of peripheral neuropathy in a total of 20.6% limbs making it the fourth most sensitive screening tool in this study, it is essential to consider the variations and lack of agreement in current literature regarding its sensitivity as a diagnostic tool.

The Neurothesiometer categorized 11.1% of limbs as being at risk of peripheral neuropathy, as determined by participants' ability to perceive vibration after 21V, as categorized by Dubey et al. (2022). A study conducted by Lanting et al. (2020), found that the neurothesiometer is the most reliable modality for the assessing vibration perception threshold.

However, sensitivity and specificity of this tool vary across studies due to the lack of standardization in gold standard tools. Despite these variations, the neurothesiometer is still considered a good screening tool for DPN and has been recommended by multiple screening guidelines (Ramanathan et al.,2021). There is also controversy regarding the cut-off point for determining the risk of neuropathy, and no consensus has been reached regarding which cut-off point should be utilized (Malik et al.,2013). The standard cut-off point for the neurothesiometer is 25V whilst other studies have graded the severity of peripheral neuropathy based on the value obtained by the VPT (Saha et al.,2011). Therefore, although 11.1% of limbs were categorized as being at risk of neuropathy in this study, it is important to consider the variation in result interpretation due to the lack of standardization. Also when comparing the result of this research to previous literature the variation in the threshold to determine if patient is at risk of neuropathy or not should be taken into consideration.

Lastly, the Semmes-Weinstein 10g monofilament reported 4% of limbs as having signs of neuropathy which is a result of absent sensation for one or more points (Mishra et al.,2017). Although various guidelines such as the IWGDF(2015), ADA (2014), NICE (2019) amongst others recommend the 10-g monofilament as a screening tool for diabetic peripheral neuropathy since its small, inexpensive and easy to use, its diagnostic value is still a subject of debate among researchers (Zhang et al.,2018). The sensitivity and specificity percentages of the monofilament test vary widely, ranging from 41% to 93% and 68% to 100% respectively (Ramanathan et al.,2021). Moreover, many studies have suggested that the monofilament test should be used in conjunction with other screening tools rather than on its own since it is suggested that the 10-g monofilament may only detect the presence of peripheral neuropathy at a later stage (Pambianco et al., 2012); (Ang et al.,2018); (Ramanathan et al.,2021). Consistent with the literature, this present research found that only 4% of limbs had absent sensation as determined by the 10-g monofilament test when compared to the 32.5% of the NC-Stat® DPN Check®.

Therefore, the outcomes of this research study confirmed variations amongst the different screening tools employed to identify DPN, suggesting inconsistencies between results. Although, the NC-Stat® DPN Check® detected the highest percentage of limbs with abnormal sensation, one can still attest that the actual incidence of DPN was not determined in the studied cohort. This is because none of the screening tools used in this research study are deemed as being gold standard tests. Therefore, it should be taken into consideration the possibility that some outcomes may yield a false negative or false positive result which can only be eliminated when using a gold standard tool such as the nerve or skin biopsy. However, these are invasive tests and are not practical for the screening of DPN at a primary care level.

Findings in this research are congruent to other studies conducted recently (Azzopardi et al.,2017); (Brown et al.,2017); (Ramanathan et al.,2021). However, other researchers obtained various outcomes, which will be explicated in the subsequent subsections. Therefore the subsequent discussion compares each subjective screening tool utilized in conjunction with the NC-Stat® DPN Check® and contrast the outcomes attained in this research investigation with prior literature.

5.2.2 Comparing the Semmes-Weinstein 10-g monofilament and NC-Stat® DPN Check®

A study conducted by Binns-hall, et al. (2018), investigated different screening tools namely the Toronto Clinical Neuropathy Score (TCNS), 10-g monofilament, NC-Stat® DPN Check® and Sudoscan for the early diagnosis of DPN and the high risk foot. According to the study findings, the prevalence of DPN was 14.4% with the 10-g monofilament and 51.5% with the the NC-Stat tool which recorded the highest prevalence. The discrepancy observed between the 10-g monofilament and the NC-Stat tool was considerable, since the sural nerve conduction test identified a significantly higher percentage of limbs with peripheral neuropathy compared to the monofilament test as was the case in this study.

Another study by Sheshah, et al. (2020), also compared the prevalence of DPN using the neuropathy disability score (NDS) which was determined as their ‘gold standard tool’ with the 10-g monofilament, the NC-Stat® DPN Check® and Sudoscan. The results showed that the 10g monofilament and NDS had a lower prevalence rate of 19.5% and 13.8% respectively, whereas the NC-Stat and the Sudoscan reported a prevalence rate of 40.9% and 73% respectively. This therefore indicates that the incidence of reporting DPN is higher with the NC-Stat when compared to 10-g monofilament. However, the study questioned whether it was possible that both the NC-Stat and the Sudoscan overestimated the prevalence rates of DPN. Although, it is important to note that the NDS was considered as the 'gold standard' in this study, this is not agreed upon by most literature. Additionally, a number of methodological flaws were noted in the study since the years of diabetes onset and the age of participants which is known to be a contributing factor to neuropathy were not reported.

Another study by Pambianco, et al. (2012), found that all participants who had a positive monofilament test also resulted to have abnormal sural nerve conduction. However, 60% of participants who resulted with present sensation when using the monofilament test were classified as having neuropathy with the NC-Stat® DPN Check®. Consequently, it can be inferred that the use of the 10-g monofilament may indicate the detection of peripheral neuropathy at an advanced stage, where complications and neuropathic symptoms may have already manifested. On the other hand, the NC-Stat® DPN Check® was able to diagnose peripheral neuropathy in its subclinical stages.

In this current study, a significant difference in results was obtained between the 10-g monofilament and the NC-Stat® DPN Check®. This can be attributed to the fact that a substantial proportion of limbs that were identified as having normal sensation with the monofilament were actually found to have mild (4.1%) or moderate (25.6%) neuropathy when tested with the NC-Stat tool, accounting for 29.7% of limbs. On the other hand, it should be noted that a fair percentage (70.3%) of limbs that were determined to have intact sensation with the 10-g monofilament also showed normal nerve conduction with the NC-Stat tool. Furthermore, although only 5 limbs were identified as insensate using the monofilament, all of these limbs were classified as mild or moderate neuropathy by the NC-Stat.

Therefore, the results of this study supports the findings from previous literature discussed above which suggests the possibility that the NC-Stat device is better in detecting DPN at an earlier stage when compared to the 10-g monofilament. This further suggests that the 10-g monofilament is able to detect peripheral neuropathy in a later stage.

5.2.3 Comparing the 128Hz Traditional tuning fork and NC-Stat® DPN Check®

A study by Brown et al. (2017), compared the NC-Stat® DPN Check® with different subjective screening tools namely 128Hz traditional tuning fork, the Norfolk Quality of Life Diabetic Neuropathy questionnaire (QOL-DN), 1-g and 10-g monofilament. Both the on/off method and the timed method for the tuning fork were utilized in the study. The results indicated that the 128Hz tuning fork alone was not accurate and therefore should be used as a tandem measure when screening. Furthermore, the timed tuning fork method showed to have no significant correlation between the tools being tested. The limitation of this study is the small cohort of participants which was a total of 34 adults as well as no indication regarding the years of diabetes onset.

The present research findings is congruent with the literature mentioned above, as a significant discrepancy was observed between the 128-Hz Tuning fork and the NC-Stat® DPN Check®, since a p-value (P=0.000) less than the 0.05 level of significance was obtained. This is attributed to the fact that the limbs categorized as having present sensation with the tuning fork, were in contrast classified as having mild (3%) or moderate (16%) neuropathy with the NC-Stat tool.

While 81% of limbs categorized as having present sensation with the tuning fork were also classified as having normal conduction with the NC-Stat tool.

Moreover, among the limbs that were reported to have absent sensation by the tuning fork, 15.4% of limbs were not in agreement since they exhibited normal conduction, while the remaining limbs showed a mild (15.4%) or moderate (69.2%) neuropathy with the NC-Stat® DPN Check®.

Hence, given the disparity between the outcomes of the 128-Hz TTF and the NC-Stat device, there are concerns about relying solely on the 128-Hz tuning fork as a diagnostic tool. This also aligns with the findings of the study conducted by Brown, et al. (2017), which also suggests caution in relying on the 128-Hz TTF.

5.2.4 Comparing the 128Hz ETF and NC-Stat® DPN Check®

This study is the first to compare the 128Hz ETF with the NC-Stat® DPN Check®, as no previous research has been published on this topic. Both the 128Hz ETF descending mode and the constant mode were used in this study and compared to the NC-Stat® DPN Check®.

In this research study, an overall significant difference was observed between the 128Hz ETF descending and the NC-Stat tool, as evidenced by the rejection of the null hypothesis. However when analyzing each category of the 128HZ ETF, a significant discrepancy was noted between the normal to mild and normal to moderate category only of the NC-Stat tool. Among the limbs, which result in the low risk category of the ETF, 77.3% had normal conduction with the DPNCheck. However, it is important to note that some limbs deemed as low risk with the ETF were reported to have mild (2.1%) or moderate (20.6%) neuropathy with the NC-Stat tool.

Discrepancies were also noted in the medium and high risk category of the ETF descending when compared with the NC-Stat device.

A significant discrepancy was obtained between the 128Hz ETF constant and the NC-Stat® DPN Check®. This study illustrated that 79.2% of limbs which classified as having present sensation by the ETF constant also had normal conduction as reported by the NC-Stat tool. However, the ETF identified 20.8% of limbs with present sensation as having mild (3.1%) or moderate (17.7%) of neuropathy with the NC-Stat modality. Among limbs with absent sensation according to the ETF, a total of 70% were classified as having mild (13.3%) or moderate (56.7%) neuropathy whilst 30% had normal sural nerve conduction.

The 128-Hz ETF is a relatively new device that displays potential in detecting peripheral neuropathy. In the current study, notable difference were observed when comparing the ETF to the other screening tools, where the findings indicate that the ETF obtained the highest number of limbs classified with peripheral neuropathy when compared to the other subjective tools. Nevertheless, additional research is required to comprehensively comprehend the effectiveness of the ETF, particularly in terms of its sensitivity and specificity for the diagnosis of DPN. Moreover, since variations were noted between the modalities recommended by screening guidelines, there is a need to consider incorporating other subjective measures into diabetic foot screening guidelines, while also focusing on standardizing the methods of the currently employed and recommended screening tools.

5.2.5 Comparing the Neurothesiometer and NC-Stat® DPN Check®

There is no current literature available that compares the neurothesiometer with the NC-Stat® DPN Check® for the diagnosis of DPN.

This research study established a significant difference between the neurothesiometer and the NC-Stat® DPN Check®, with the alternative hypothesis being accepted due to obtaining a P-value of less than 0.05 level of significance. Furthermore, when analyzing the categories in depth, this study also identified a significant difference between the normal to moderate neuropathy group of the NC-Stat when compared with the neurothesiometer. Specifically, 75% of limbs classified as low risk (< 21V) by the neurothesiometer were also deemed as having normal sural nerve conduction with the NC-Stat device. However, some limbs that were reported as low risk with the neurothesiometer, exhibited mild (5.4%) or moderate (19.6%) neuropathy with the nerve conduction tool. Discrepancies were also noted in the medium risk category of the neurothesiometer (21V- 30.99V) when compared with the NC-Stat® DPN Check®. Notably, no limbs were classified as high risk (> 31V) for neuropathy with the neurothesiometer; therefore this category could not be measured and compared.

The study found that the majority of limbs categorized as low risk of neuropathy by neurothesiometer exhibited normal nerve conduction by the NC-Stat device. Also, those categorized as medium risk of neuropathy by the neurothesiometer were mostly classified as having mild or moderate neuropathy by the NC-Stat tool. These findings suggest a pattern of similarities between the results obtained by the neurothesiometer and the NC-Stat® DPN Check®. However, it is essential to acknowledge that certain limbs did not exhibit identical outcomes when compared with the screening tools therefore indicating a significant difference.

Furthermore, this study may suggest the importance in considering the lack of standardization in VPT cut-off points, which may hinder true comparison between studies. Additionally, as previously mentioned these findings underscore the importance of using the neurothesiometer in combination with other screening modalities to achieve better outcomes in detecting DPN.

5.3 The importance of objective measurement vs. subjective measurement for the diagnosis of DPN

This research study has highlighted a significant difference in the test results between the subjective tools and the objective tool utilized for the detection of DPN. While most diabetes foot screening guidelines such as the ADA (2014), IWDGF (2014), NICE (2019), amongst others primarily recommend the use of subjective tools for detecting peripheral neuropathy, the incorporation of an objective tool could offer potential benefits as has been highlighted in this study.

The findings of this study emphasize the need of utilizing multiple screening tools when assessing for DPN and incorporating both subjective and objective measures to ensure correct diagnosis. By employing a variety of screening tools during clinical assessment including both subjective and objective measures, clinicians can ensure a more comprehensive assessment of peripheral neuropathy. Implementing this approach can contribute to reducing the number of patients who may go undiagnosed or receive incorrect diagnosis of DPN.

Furthermore, since different screening tools have varying levels of sensitivity in detecting peripheral neuropathy, by utilizing multiple screening tools, clinicians can enhance the sensitivity of the screening process. This means that it is more likely to identify cases of peripheral neuropathy even in its early stages which might be missed when utilizing certain screening modalities.

Additionally, employing multiple screening tools also allows for the validation and cross-referencing of results. When different screening tools consistently yield similar results as has been highlighted in various studies (Azzopardi et al.,2017); (Ramanathan et al.,2021), it increases confidence in the correct diagnosis. On the other hand, discrepancies between tools indicates the need for further investigations and additional testing as well as prompt referral for further neurological testing.

Subjective tools, which include patient-reported symptoms and interpretation of test utilized, provide valuable insights into the presence of peripheral neuropathy (Gewandter et al.,2016). However, one should keep in mind that subjective measures depend on the patient's response which can introduce potential limitations such as possibly overestimating or underestimating the test outcome, leading to false positive or false negative results. Additionally, if the patient fails to fully comprehend the procedure, it can result in inaccuracies in the obtained results. On the other hand, objective tests such as NCS, provide measurable data on nerve function and objectively assess the extent of peripheral neuropathy. By combining subjective and objective modalities, a more comprehensive understanding of peripheral neuropathy can be achieved, allowing for timely treatment and better management strategies with effective secondary risk factor control (Medrano & del Mar Gili, 2022).

In conclusion, the utilization of multiple screening tools, along with subjective and objective modalities, has shown to be imperative in evaluating peripheral neuropathy comprehensively. This approach may improve sensitivity in diagnosis, validates results, encompasses the diverse aspects of neuropathy and accommodates individual variations in symptom presentation. By adopting this comprehensive methodology, healthcare professionals can provide more effective management strategies and improve the overall outcomes for individuals living with peripheral neuropathy. Furthermore, when results do not concur more neurological evaluation should be conducted to establish a better understanding.

5.4 Evaluating current diabetic foot screening guidelines: exploring advancements in technology for improved diagnosis and existing methods

Complications affecting the lower extremities due to diabetes pose a substantial burden on individuals with the condition. The incidence of limb loss resulting from diabetes-related complications remains unacceptably high, with a limb being lost worldwide every 20 seconds due to this cause (Armstrong et al.,2017). This alarming statistic suggests that the current management of diabetic foot conditions may not be effectively tackling the issue, highlighting the urgent need for the implementation of new and efficient strategies. Accurate diagnosis and timely referral of peripheral neuropathy through diabetic foot screening guidelines play a crucial role in reducing the risk of developing complications such as ulcerations.

Current guidelines recommend subjective tools which have been widely adopted in clinical practice for assessing patients' risk and detection of peripheral neuropathy. However, the variations and low level of evidence supporting these recommendations underscore the need for improved screening methods and guidelines.

In this research study, the variability in outcomes when comparing different screening tools used in a clinical setting raises questions about the possibility of improving the current diabetic foot screening guidelines. The considerable disparity among the subjective tools utilized in this study to identify limbs with peripheral neuropathy, along with variations in results when compared to the objective measure of the NC-Stat device, highlights a concern regarding the potential false positive or false negative result. These findings emphasize the importance of standardizing guidelines to address the variation among screening tools.

Therefore, when screening tools recommended by diabetic foot screening guidelines yield inconsistent results for detecting peripheral neuropathy, it creates challenges. Discrepancies among these tools can lead to confusion and uncertainty in accurately detecting and classifying peripheral neuropathy in its early stages in individuals with diabetes. Healthcare professionals may face dilemmas in selecting the most reliable and valid screening tool for accurate diagnosis. Furthermore, the variations among screening tools can also affect the consistency of data collection and research studies, making it challenging to compare and generalize findings across different settings.

Efforts should be made to address these discrepancies and establish consensus on the most effective neuropathy screening tools. Collaborative research, technological advancements and evidence-based practices can improve the reliability and consistency of screening methods, ultimately leading to better diagnosis, management and prevention of DPN.

Innovative diagnostic tools and technological advancements offer promising alternatives for more effective and objective measures such as the NC-Stat® DPN Check® which in this study has shown to have the highest sensitivity of detecting peripheral neuropathy. Therefore, new modalities have the potential to enhance the detection and early intervention of DPN, thereby improving patient outcomes. Thus, a paradigm shift toward incorporating these emerging screening tools into revised guidelines is necessary to ensure optimal and evidence-based care for individuals with diabetes.

For instance, Dubey et al. (2022), developed a software that generates a risk assessment tool based on patient data to predict the level of risk for peripheral neuropathy. This is particularly valuable in situations where screening devices or trained professionals are not readily available, making it challenging to accurately determine the severity of neuropathy. According to the authors, the software utilized patient's clinical parameter which has demonstrated an acceptable level of accuracy, with performance expected to improve as high-quality data are gathered over time. The software is likely to yield even better results with a larger dataset for training. Moreover, this new software is user-friendly, not time consuming and can be easily implemented in a clinical setting which is a crucial factor to consider when selecting screening tools for successful implementation.

The emergence of new technologies opens up possibilities, including the use of artificial intelligence (AI) where incorporating AI-driven tools and algorithms into the screening process can enhance its efficiency. However, further research is necessary to determine which technologies have the greatest potential for accurately diagnosing peripheral neuropathy especially in is its subclinical stages. These advancements have the potential to revolutionize the field, providing healthcare professionals with improved tools and approaches for the early detection and management of DPN.

5.5 Clinical Relevance

The findings of this research study have important implications for current clinical practices and guidelines, as well as future research. This research has demonstrated substantial differences in the detection of peripheral neuropathy when utilizing different screening modalities. This implies that just relying on a single screening method to detect peripheral neuropathy in patients with type 2 diabetes mellitus during clinical practice may result in a significant number of false positive or false negative results, leading to inaccurate diagnosis of DPN and potential risk to patient's health. Therefore, it is advisable to consider using multiple screening modalities together with a detailed clinical evaluation for better diagnosis and treatment plan. Moreover, when results of various tests do not concur, further more accurate diagnostic tests should be made to ensure an accurate diagnosis and prompt care.

The increase in amputations worldwide (Armstrong et al.,2017) may be attributed to the inaccurate or delayed diagnosis of DPN which is a major risk factor of amputations. This can potentially be caused by inconsistencies amongst different screening modalities commonly used for the initial assessment of neuropathy at a primary care level. This can pose a significant threat to high-risk patients who may be falsely diagnosed as not having peripheral neuropathy, depriving them of early and effective treatment to prevent further deterioration. Additionally, this may delay any further investigations required to determine the full extent of this condition. Therefore, it is crucial to ensure accurate and timely diagnosis to slow down the progression of peripheral neuropathy and take appropriate precautions to protect the skin integrity of the foot.

This further highlights the importance of standardized diabetes foot screening guidelines to ensure that all healthcare providers are well informed on the pros and cons of certain test modalities and to ensure an effective approach to assess patients' risk of developing peripheral neuropathy. This is particularly crucial because various commonly used screening tools have reported conflicting results, leading to inconsistencies in the diagnosis of peripheral neuropathy.

Furthermore, accurate diagnosis of peripheral neuropathy at a primary care level can also help to reduce unnecessary referral for secondary screening or treatment which can lead to long waiting lists and delayed care for patients who genuinely require attention. By avoiding such referral for patients who do not have peripheral neuropathy, healthcare providers can prioritize prompt care for those who do actually require it, ultimately benefiting the patient and the healthcare system as a whole.

However, in cases where results of clinical testing do not concur, close monitoring and prompt reporting are crucial. Regular reviews are also recommended, with the frequency of screening determined by the severity of the patient's risk status.

Additionally, more accurate methods of assessment such as conventional NCS can be used to resolve any conflicting results that may arise from the use of different clinical tools. However, such modalities may not be readily available or considered too costly for screening purposes. For this reason, the NC-Stat® DPN Check® was utilized in this study as it demonstrated to be a reliable and accurate non-invasive test for screening diabetic peripheral neuropathy due to its ability to produce objective data and categorize the severity of the condition. Furthermore, it has shown to have advantages over other subjective screening tests, as it provides objective data on nerve conduction velocity and is not influenced by patient-reported symptoms or subjective interpretation by the clinician (Sharma et al.,2015). This further demonstrates the importance of including objective screening especially when further evaluation is required.

Therefore, patients diagnosed with DPN should receive appropriate treatment and preventive measures to reduce the risk of further deterioration. This may involve lifestyle advice to control risk factors and implementation of any required treatment interventions.

5.6 Recommendations of Clinical Practice

Currently, local Standard Operating Procedure (SOP) for diabetic foot screening in Primary health care suggests the use of the 10g monofilament and the 128Hz traditional tuning fork for the detection of peripheral neuropathy. Following examination, when neuropathy is diagnosed, the SOP provides a set of evidence based recommendations such as general foot care advice, therapeutic footwear/ orthotics and further biomechanical analysis to prevent complications.

However, in the primary care setting, healthcare professionals are unable to refer patients for any additional screening assessments as there is no specialized clinic available to provide further evaluation for a more accurate diagnosis of peripheral neuropathy particularly in its early stages when symptoms may not be present. This study has shown that a combination of multiple screening methods can significantly improve the accuracy of diagnosis and enable the development of more effective treatment plans for peripheral neuropathy. Establishing a specialized neuropathy clinic that utilizes both subjective and objective screening tools has shown to be important and recommended to produce the best outcomes (Jimenez & Gili Rius, 2022). Hence, this study recommends such a clinic to be introduced at primary care clinics in Malta to help address DPN more effectively.

In addition, by allocating more time for screening, this specialized clinic can ensure that patients receive a comprehensive evaluation that takes into account their medical history, symptoms and physical examination findings. This will therefore provide a more precise diagnosis and enable the development of a tailored treatment plan as well as providing education and support.

Furthermore, a specialized neuropathy clinic can offer access to a multidisciplinary team who bring their unique expertise to the evaluation and management of peripheral neuropathy. This team approach can help ensure that patients receive a comprehensive assessment and personalized treatment plan as well as any necessary support to manage their condition effectively in the aim to slow progression of neuropathy.

5.7 Critique of this Study

No study can be completely flawless as every type of research comes with inherent strengths and weaknesses. Therefore, researchers must acknowledge the potential limitations and sources of error in their studies and take measures to minimize them, while also being transparent about their limitations and potential impact on the study findings (Olufowote, 2017).

Furthermore, it is important that limitations in the study design are pointed out so that future research can formulate an improved study model (Dennhardt, 2014).

Although all reasonable effort was made to ensure that an optimal methodology was designed and implemented to this study, the following points highlight the limitations observed.

5.7.1 Sample Size

The sample size of this study was one of the main limitations as from the 84 individuals who fit the inclusion criteria, only 63 accepted to participate and therefore a total of 126 limbs were assessed with all five screening tools. The sample size was somewhat small mainly due to rigorous exclusion criteria which limited the number of potential participants. The purpose of having such specific criteria was to control for external variables known to affect neuropathy risk such as age, oedema, vascular compromise, year of diabetic onset amongst others (Brown et al.,2017) (Brisset & Nicolas, 2018).

Furthermore, the exclusion criteria were also intended to safeguard participants from any harm by taking part in the assessment process. For example individuals with implanted electronic devices were not able to participate due to the electrical stimulation produced by the NC-Stat (Abe, et al., 2021).

Additionally, small sample sizes can increase the risk of sampling bias, as the sample may not be representative of the population of interest, which can affect the generalizability of the study findings (Andrade, 2020).

5.7.2 Screening Modalities

Another possible limitation in this study is that all the screening modalities utilized in this study for the assessment of peripheral diabetic neuropathy are not considered “gold standard”. Literature illustrates that large nerve fiber function are affected in a later stage than small nerve fibers in diabetic peripheral neuropathy (Halpern et al.,2013). Therefore, although the NC-Stat® DPN Check® has shown to potentially detect early stages of peripheral neuropathy, it only quantitatively measures large nerve fiber function namely the sural nerve (Pafili et al., 2017). The “gold standard” test for diagnosing peripheral neuropathy is a lower limb nerve biopsy which is deemed as a time consuming and invasive procedure which is not recommended for routine screening in primary care setting (Carmichael et al.,2021). Nevertheless, despite the ongoing debate surrounding diagnostic tools for DPN, the NCS have been widely acknowledged by numerous researchers as the non-invasive gold standard for early stage diagnosis. However, this test is not usually readily available in primary care setting due to it being time-consuming and requires specialized practitioners (Sharma et al.,2015).

Furthermore, the results obtained in this study should be interpreted with caution as there were significant differences in the number of limbs between categories of each screening tool.

5.7.3 Patient Compliance

Four of the screening tools used namely the Semmes-Weinstein 10g monofilament, 128-Hz TTF, neurothesiometer and 128- Hz ETF are all subjective tests. Some participants exhibited difficulty in understanding the testing method especially when instructed to report the initial or diminished vibration perception produced by the tool. When faced with such challenges, the researcher repeated the explanation in detail again and placed the stimulus on the participant's hand to familiarize the patient with sensation produced by the tool.

Failure to properly understand the procedure could have led to error in the results obtained. Therefore, to decrease the margin of error, every screening test was repeated 3 times on each foot and a mean result was recorded.

5.7.4 Selection of Screening Tools

There are many screening modalities available which clinicians can utilize to detect the presence of peripheral neuropathy. The screening modalities were chosen based not only on their recommendation in various diabetic foot screening guidelines but also on their availability in the clinics were the study was conducted. Moreover, although screening tools have different methods of application, and their recommendations of use are varied in foot screening guidelines, the ones chosen in this study were deemed as valid, evidence based and the most highly recommended for DPN screening and diagnosis.

5.8 Recommendations for future research

This study has contributed to the body of knowledge with regards to screening modalities that can be utilized in primary care settings to detect peripheral neuropathy in people living with diabetes mellitus. However, as with any research, there are still areas that warrant further investigation. Future research could confirm the findings of this study and expand upon them by providing additional data and information beyond the scope of this study.

The recommendations are as follows:

- To strengthen the statistical evidence, this study could be replicated using a larger sample population. The results of both studies could then be compared to either confirm or contradict the findings of this study.
- For further validation of findings, this study could be repeated by comparing the same screening tests to a gold standard reference used for diagnosing peripheral neuropathy such as NCS
- The diagnostic techniques utilized in this study can be compared to other screening tools for peripheral neuropathy such as the Michigan Neuropathy Screening Instrument (MNSI), Diabetic Neuropathy Symptom Questionnaire (DNS), pin-prick test and the reflex test as well as other valid modalities.

5.9 Plans for Dissemination of Findings

The dissemination of this research study will occur in the following manner:

- An article for potential publication in a peer-reviewed journal.
- A paper disseminated to all the primary care stakeholders, outlining the research's findings.
- Consultation/meetings with management and Primary healthcare officials to discuss the implementation of a specialized neuropathy clinic within a primary setting. This would be a first in Malta since to date no specialized neuropathy foot clinic is available at a primary care level for referral when peripheral neuropathy is suspected by health care professionals. Therefore, in such cases, additional screening modalities including objective testing may be carried out in specialized clinic to facilitate a comprehensive evaluation and enhance management of peripheral neuropathy.

Chapter Six

Conclusion

Peripheral neuropathy is one of the most common chronic progressive complications in type 2 diabetes mellitus, affecting approximately 50% of the adult diabetic population. (Hicks & Selvin, 2019). This consequently highlights the importance of early screening for DPN in order to reduce risk of comorbidities such as ulceration and amputation as well as sustain a good quality of life and decrease medical expenditure (Ramtahal, et al., 2015).

However, screening for DPN is challenging, due to the on-going debate on which screening tool is the most ideal to use in a primary care setting. Moreover, the lack of consensus between diabetic screening guidelines for DPN, has led to variations in practice and potentially suboptimal patient outcomes. Therefore, standardized and evidence-based guidelines are needed to improve clinical decision-making and ensure consistent and effective screening for DPN (Weck et al.,2013). Furthermore, for guidelines to be effective in a clinical practice, the screening tools recommended should be non-invasive, easily accessible and inexpensive as well as able to provide timely and accurate results (Maxim et al.,2014).

Numerous diabetic foot screening guidelines propose different tests and pathways to identify the presence of peripheral neuropathy which has created confusion among clinicians regarding which screening test to use in clinical practice. Moreover, changes in disease progression patterns, outcomes and advancements in technology for measurement and treatment call for an update in diabetic foot screening guidelines. There is an urgent need to re-evaluate and update screening modalities in light of emerging instruments and techniques. Innovative diagnostic tools and technological advancements offer promising alternatives, such as the NC-Stat® DPN Check® or risk assessment software tool from the study Dubey et al.,(2022). Therefore, incorporating these emerging screening tools into revised guidelines is necessary to ensure advanced evidence-based care for individuals with diabetes.

In this study, the objective measure NC-Stat® DPN Check® was compared with subjective screening tools recommended in literature and used for the detection of peripheral neuropathy since it demonstrated to have a good correlation with the standardized NCS (Shibata et al.,2019). The NC-Stat tool offers advantages over other subjective screening modalities, since it provides objective data where the results are not dependent on patient-reported symptoms and are not subject to interpretation by the clinician.

Different non-invasive subjective screening modalities that are utilized in a primary care context, as recommended by various foot screening guidelines, were evaluated in this study for the assessment of peripheral neuropathy in patients living with type 2 diabetes mellitus. The modalities included the Semmes-Weinstein 10-g monofilament, 128-Hz traditional tuning fork, 128-Hz electronic tuning fork and the neurothesiometer. When compared with the NC-Stat® DPN Check®, a significant difference was observed between all the subjective screening tools mentioned, although the strength of association varied among them. This suggests that some instruments are more sensitive in the detection of DPN than others which can lead to patients being wrongly diagnosed or misdiagnosed with peripheral neuropathy. Therefore, these findings suggest the importance of utilizing multiple modalities for screening of DPN in order to gain a correct comprehensive understanding of the patient's neurological status and develop a more effective treatment plan.

Moreover, following comparison of all the screening tools used, the NC-Stat showed the highest percentage of limbs with the presence of peripheral neuropathy. This may suggest that in cases where subjective screening tools used in routine diabetic screening yield conflicting results, incorporating the NC-Stat® DPN Check® as an objective tool for further evaluation may help in potentially detecting sub-clinical peripheral neuropathy.

Therefore these findings highlight the importance of raising awareness among healthcare professionals and researchers regarding the need to cautiously interpret the results of subjective screening modalities which are very often used in primary care clinics for the detection of peripheral neuropathy and also happen to be the screening modalities advocated in diabetic foot screening guidelines.

This further accentuates the urgent need to identify the true early biomarker of nerve degeneration for the diagnosis of DPN, in order to ensure the best clinical care. Therefore, more rigorous and robust studies comparing various screening tests to a gold standard tool used for the diagnosis of DPN are warranted to provide evidence with the goal of determining the most valid non-invasive screening modality for peripheral neuropathy. This could help to reduce the proportion of individuals who may receive a false negative or false positive diagnosis for peripheral neuropathy, leading to potentially denying any preventative care and delaying further screening and treatment. Consequently, concerted global efforts amongst the clinical, scientific and research community should be directed towards addressing these limitations and strive in developing more standard screening methods and early detection strategies to effectively reduce the prevalence of complications in DPN and its complications with the aim of saving limbs, saving lives.

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Appendices

Appendix 1- Permission Letter from Head of the Podiatry Department, Faculty of Health Science, University of Malta

Letter of Permission to the Head of Podiatry Department

Stephanie Pleven

No objection

17.12.2020

Prof. Cynthia Formosa
Head of Podiatry Department
University of Malta

Date: 11th December 2020

Dear Prof. Formosa,

I am a post-graduate student reading for a M.Sc. in Podiatry (Research) at the University of Malta. I will be conducting a dissertation entitled **'A Comparison of Screening Tools for the accurate diagnosis of Peripheral Neuropathy in Type 2 Diabetes'.**

The purpose of this study is to compare different screening modalities utilized for the detection and accurate diagnosis of Peripheral Diabetic Neuropathy in a Primary Care when compared to the nerve conduction test (NC-Stat DPN check). This study aims to give a better understanding to which tool will correctly diagnose Peripheral Diabetic Neuropathy in its early stage to prevent future complications and co-morbidities.

Participants in this study will be examined using different screening modalities for PDN such as 10g monofilament, 128Hz tuning fork, neurothesiometer, VibraTip and the NC-Stat DPN check.
A total time of approximately 1 hour is required for data collection and participants only need to attend once. . Each individual will be informed by the intermediary, Mr. Matthew Schembri on what the study entails.
Prior to data collection, each individual will be provided with an information letter along with a consent form which will be required to be signed.

Individuals are required to fit the following inclusion criteria in order to participate in this study:

- One hundred Participants both male and female
- Individuals attend the podiatry clinic at a Primary Health Care Centre
- Above 18 years of age
- Adults diagnosed with type 2 diabetes mellitus for at least 10 years
- No history of neurological problems other than neuropathy such as nerve root compression and cerebral vascular disease & no history of hypothyroidism
- No history of alcoholism or diagnosed with alcoholic liver disease
- No use of recreational drugs or illegal substances
- Participants with implanted electronic devices will be excluded
- No lesions, broken skin or wounds on the tested area

I would like to ask for your permission to make use of the neurothesiometer and NC-Stat DPN check available in the Podiatry Biomechanics Lab at the Faculty of Health Science A total time of approximately 1 hour will be taken for examination to be completed and each participant will be seen only once for data collection. Participants are required to attend the Podiatry clinic at Primary Health Care Centre where the participant was recruited from for data collection.

This research study still awaits Ethical approval from the Faculty or University Research Ethics committee, although departmental approval has already been attained.

For any further information regarding this research study, please do not hesitate to contact myself or my supervisor on our phone numbers as listed below.

I would like to take this opportunity to thank you in advance whilst I await your reply.

Best Regards,
Stephanie Pleven

Investigator's Contact Details:
Stephanie Pleven

Supervisor's Contact Details:
Prof. Cynthia Formosa

Appendix 2- Permission Letter from Professional Lead of Podiatry Services

Letter of Permission to Professional Lead of Podiatry Services

Stephanie Pleven

Mr. Andrew Scicluna
Professional Lead of Podiatry Services
Podiatry Department – Birkirkara Health Centre

Date: 11th December 2020

no objection

Andrew Scicluna
Professional Lead
Podiatry Department

Dear Mr. Scicluna,

I am a post-graduate student reading for a Msc. in Podiatry (Research) at the University of Malta. I will be conducting a dissertation entitled **'A Comparison of Screening Tools for the accurate diagnosis of Peripheral Neuropathy in Type 2 Diabetes'.**

The purpose of this study is to compare different screening modalities utilized for the detection and accurate diagnosis of Peripheral Diabetic Neuropathy in a Primary Care when compared to the nerve conduction test (NC-Stat DPN check). This study aims to give a better understanding to which tool will correctly diagnose Peripheral Diabetic Neuropathy in its early stage to prevent future complications and co-morbidities.

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- Adults diagnosed with type 2 diabetes mellitus for at least 10 years
- No history of neurological problems other than neuropathy such as nerve root compression and cerebral vascular disease & no history of hypothyroidism
- No history of alcoholism or diagnosed with alcoholic liver disease

- No use of recreational drugs or illegal substances
- Participants with implanted electronic devices will be excluded
- No lesions, broken skin or wounds on the tested area

I would kindly like to ask for your permission to recruit 100 individuals above 18 years of age with type 2 Diabetes Mellitus who attend the Podiatry clinic within the Primary Health Care Centres to participate in this research study. These participants will be required to attend the Podiatry clinic at Primary Health Care Centre where the participant was recruited from for data collection.

This research study still awaits Ethical approval from the Faculty or University Research Ethics committee, although departmental approval has already been attained.

For any further information regarding this research study, please do not hesitate to contact myself or my supervisor on our phone numbers as listed below.

I would like to take this opportunity to thank you in advance whilst I await your reply.

Best Regards,
Stephanie Pleven

Investigator's Contact Details:
Stephanie Pleven

Supervisor's Contact Details:
Prof. Cynthia Formosa

Appendix 3- Permission Letter from the Data Protection Officer

Primary HealthCare

FRN 1940

Website: http://www.health.gov.mt

PRIMARY HEALTHCARE

7 Harper Lane,
Floriana
FRN 1940

Telephone: +356 21239993
Telefax: +356 21222856

14 October 2021

Stephanie Pleven

Re: Your request to carry out a study within the Primary Health Department

Dear Ms Pleven,

I am pleased to inform you that your request to carry out the research within the department has been **fully approved**.
May I inform you that as we have to abide to the Data Protection Law, **we cannot provide you with a list of data subjects' (clients/patients/staff) personal contact details**.* The data subjects also have to sign an informed consent form that also includes a data protection statement (unless it is an anonymous questionnaire) prior to participating (see E below). Any modifications of this approach would have to be first discussed with the data protection officer. Where statistics are involved, only data in terms of age, sex etc can be forwarded to you but not names of individuals.

May I bring to your attention that the researcher is obliged to apply necessary safeguards as a condition for carrying out this research, namely -

A. The personal data (of data subjects) accessed or given are only to be used for that specific purpose to conduct the research and for no other purpose;

B. At the end of the research, all personal data should be destroyed;

C. All references to personal data should be omitted in the report unless an informed consent is specifically obtained from the person being identified in the research report;

D. Participation in the research being conducted should be at the discretion of the individual, and they can refuse any participation whatsoever if they so wish;

E. If data subjects (patients/staff) are going to be interviewed, video recorded or given a non-anonymous questionnaire to fill, an informed consent form should be signed by the participating data subject and a privacy policy statement read to them; Faces should be hidden or digitally modified as to conceal identity;

F. Any other measure deemed fit by the respective Head, depending on the research to be carried out.

I sincerely wish you every success in your studies.

Yours truly,

Dr Mario Vella, Data Protection Officer, Primary HealthCare
f/ CEO, Data Controller, Primary HealthCare

**May I suggest that you offer the invitation for participation through any officer in charge (e.g. Nursing officer/Senior GP/service provider)*

Appendix 4- Letter to Intermediary – Podiatrist

Letter to Podiatrist- Intermediary

No objection to act as intermediary

Stephanie Pleven

Mr Matthew Schembri
Podiatrist
Primary Health Care

Date: 11th December 2020

Dear Mr. Schembri,

I am a post-graduate student reading for a Msc. in Podiatry (Research) at the University of Malta. I will be conducting a dissertation entitled **'A Comparison of Screening Tools for the accurate diagnosis of Peripheral Neuropathy in Type 2 Diabetes'.**

The purpose of this study is to compare different screening modalities utilized for the detection and accurate diagnosis of Peripheral Diabetic Neuropathy in a Primary Care when compared to the nerve conduction test (NC-Stat DPN check). This study aims to give a better understanding to which tool will correctly diagnose Peripheral Diabetic Neuropathy in its early stage to prevent future complications and co-morbidities.

Participants in this study will be examined using different screening modalities for PDN such as 10g monofilament, 128Hz tuning fork, neurothesiometer, VibraTip and the NC-Stat DPN check. A total time of approximately 1 hour is required for data collection and participants only need to attend once.
Prior to data collection, each individual will be provided with an information letter along with a consent form which will be required to be signed.

Individuals are required to fit the following inclusion criteria in order to participate in this study:

- One hundred Participants both male and female
- Individuals attend the podiatry clinic at a Primary Health Care Centre
- Above 18 years of age
- Adults diagnosed with type 2 diabetes mellitus for at least 10 years
- No history of neurological problems other than neuropathy such as nerve root compression and cerebral vascular disease & no history of hypothyroidism
- No history of alcoholism or diagnosed with alcoholic liver disease
- No use of recreational drugs or illegal substances
- Participants with implanted electronic devices will be excluded
- No lesions, broken skin or wounds on the tested area

I would highly appreciate it, if you could kindly act as an intermediary whose role is to approach potential participants and determine whether they would voluntarily participate in this study. It would also be your role to provide the information letter to these prospective participants prior to signing the informed consent.

This research study still awaits Ethical approval from the Faculty or University Research Ethics committee, although departmental approval has already been attained.

For any further information regarding this research study, please do not hesitate to contact myself or my supervisor on our phone numbers as listed below.

I would like to take this opportunity to thank you in advance whilst I await your reply.

Best Regards,
Stephanie Pleven

Investigator's Contact Details:
Stephanie Pleven

Supervisor's Contact Details:
Prof. Cynthia Formosa

Appendix 5- Approval from FREC & UREC for commencement of Study

10/11/21, 10:37 AM University of Malta Mail - UREC FORM V_15062020 7718 Stephanie Pleven

L-Università ta' Malta

Stephanie Pleven <stephanie.degiorgio.11@um.edu.mt>

UREC FORM V_15062020 7718 Stephanie Pleven

Rita Pace Parascandalo <rita.pace-parascandalo@um.edu.mt> 23 April 2021 at 08:25
To: Stephanie Pleven <stephanie.degiorgio.11@um.edu.mt>
Cc: Research Ethics HEALTHSCI <research-ethics.healthsci@um.edu.mt>, Cynthia Formosa <cynthia.formosa@um.edu.mt>

Dear Stephanie,

the amendments requested by UREC-DP have been reviewed and are verified by FREC. Approval obo FREC is granted and you may proceed with collecting data for your study.

Good luck

Regard
Dr Rita PP

L-Università ta' Malta

Dr Rita Pace Parascandalo PhD (UClan)
BSc(Hons) (Melit.), MSc(Melit.), RM

Senior Lecturer, Department of Midwifery

Chairperson, Faculty Research Ethics Committee

Faculty of Health Sciences
Office No. 48
+356 2340 1176
rita.pace-parascandalo@um.edu.mt

Appendix 6- Information Letter in English

L-Università ta' Malta

Participants` Information Sheet

Dear Participant,

My name is Stephanie Pleven and I am a post-graduate student reading for a M.Sc. in Podiatry (Research) at the University of Malta. As part of my course requirements, I am conducting a dissertation, entitled **'A Comparison of Screening Tools for the accurate diagnosis of Peripheral Neuropathy in Type 2 Diabetes'** under the supervision of Prof. Cynthia Formosa. This letter is to invite you to participate in this research study.

Before deciding to take part, it is important to fully be aware of the implication of this study and its involvement. The participant is kindly asked to read the information listed below and if any queries or any further information is required, please do not hesitate to ask me. May I take this opportunity to thank you for reading the following.

The aim of this study is to compare and confirm the accurate diagnosis of diabetic peripheral neuropathy using different commonly used screening tools when compared to nerve conduction testing using NC-Stat DPN check. Your participation in this study would help us gain a better understanding of which screening tool can reach an accurate and early diagnosis of Diabetic Peripheral Neuropathy. It is extremely important to properly diagnose Peripheral diabetic neuropathy at an early stage as it can prevent future complications and co-morbidities.

Furthermore, all data collected from this research shall be used solely for the purpose of this study.

You are being invited to participate in a study where you will be tested using different non-invasive screening tools for Peripheral Diabetic Neuropathy such as a 10g monofilament, 128Hz Tuning fork, Neurothesiometer, VibraTip ,and the NC-Stat DPN check. If you agree to participate, you will meet the researcher Stephanie Pleven once, at the podiatry clinic where the participant was recruited from. The total time for data collection is approximately 1 hour.

During the visit I, as the researcher will:

1. Ask demographic data and other general questions such as age, weight, height, and medical history
2. Assess both feet by using various non-invasive tests as previously mentioned to check for Peripheral Diabetic Neuropathy such as the 10g monofilament, 128Hz Tuning fork, Neurothesiometer, VibraTip, and the NC-Stat DPN check.

Participation in the study will be completely voluntary therefore you can accept or refuse to take part and you are not obliged to answer all the questions. You are free to withdraw at any time during the study by contacting the Researcher or Supervisor using the contact details provided below, without needing to provide any explanation and without having any negative repercussions. Should you wish to withdraw, any of the data collected from your examination will be deleted and omitted from the study.

Throughout the entire study, the data collected will remain confidential and pseudonymised by storing in code. Individuals will not be identifiable when results are published in any public domain, reports, or presentations. All data collected will be pseudonymized meaning that the data will be assigned codes and that this data will be securely stored and separately from any codes and personal data. This data may only be accessed by the researcher. The academic supervisor and the examiners will typically have access to coded data only. There may be exceptional circumstances that allow the supervisor and examiners to have access to personal data too, for verification purposes. The data files will be stored on the researcher`s personal computer that is password protected and in an encrypted format. Any material in hard-copy form will be placed in a locked cupboard.

In the event that you feel distressed due to participation in this study, the service of a healthcare professional will guide you to contact Richmond Organization which will be available at no financial cost on your part. You can contact Richmond Organization by either phoning on 21224580 or via email on 'info@richmond.org.mt'. If you choose to participate, please note that there is no direct benefit and there are no known or anticipated risks of harm. Although by participating in this study you will undergo a thorough examination for Diabetic Peripheral neuropathy.

A copy of the information sheet and consent form will be provided for future reference. As a participant, you have the right, under the General Data Protection Regulation (GDPR) and national legislation that implements and further specifies the relevant provisions of said regulation, to access, rectify, and where applicable ask for the data concerning you to be erased. Once the study is completed and the results are published, the data will be retained in anonymous form. Any personal details will be destroyed.

This study has been approved by the Research Ethics Committee of the Faculty of Health Sciences at the University of Malta.

Thank you for your time and consideration. Should you have any questions or concerns do not hesitate to contact me (Researcher) or the Research Supervisor on the contact details provided below.

Yours Sincerely,

Ms. Stephanie Pleven
Researcher

Prof. Cynthia Formosa
Research Supervisor

Appendix 7- Information Letter in Maltese

L-Università ta' Malta

<u>Formula ta' Informazzjoni għall-Parteċipanti</u>

Għażiż/a Parteċipant/a,

Jiena Stephanie Pleven, fil-preżent qed M.Sc. in Podiatry (Research) fl- Universita' ta' Malta. Bħala parti mir-rekwiżiti tal-kors, qed nagħmel riċerka bit-titlu, **'A Comparison of Screening Tools for the accurate diagnosis of Peripheral Neuropathy in Type 2 Diabetes'** taħt is-superviżjoni ta' Prof. Cynthia Formosa.

Qabel ma' tasal għal deċizjoni, huwa importanti li tifhem x'jimplika u x'jinvolvi s-sehem tiegħek f'dan l-istudju. Jekk jogħġbok, aqra sew l-informazzjoni t'hawn taħt u jekk ikun hemm xi mistoqsijiet, tiddejjaqx issaqsi lili għal aktar informazzjoni. Nixtieq nieħu din l-opportunita' biex nirringrazzjak għall-ħin li ħa tiddedika biex taqra li ġej.

L-għan ta' dan l-istudju hu li jiġi ikumparat u ikkonfermat liema metodu tat-ttestjar għad-dijanjosi tan-newropatija periferali ikkawżata mid-dijabete jipprovdi dijanjosi aktar preċiża meta imqabbel mal- itesstjar tal-konduzzjoni tan-nervituri mill-użu ta' 'NC-Stat DPN check'. Is-sehem tiegħek f'dan l-istudju jista' jgħin biex ikollna aktar għarfien dwar liema huwa l-aħjar metodu biex tilħaq dijanjosi preciża u l-aktar kmieni ta' newropatija periferali fid- dijabete biex nipprevenu kumplikazzjonijit u ko-morbożitajiet futuri. Kull informazzjoni miġbura tintuża biss għall-għan jew l-għanijiet ta' dan l-istudju.

Bħala parteċipant/a inti se tintalab tieħu sehem f'dan l-istudju sabiex ninvestigaw diversi metodi tat-ttestjar mhux invażivi bħal '10g monofilament', '128Hz Tuning fork', 'Neurothesiometer', 'VibraTip' u 'NC-Stat DPN check' għad-dijanjosi tan-newropatija periferali ikkawżata mid-dijabete Jekk taċċetta li tieħu sehem inti tintalab sabiex tiltaqa' mar- riċerkatriċi Stephanie Pleven għal darba u l- ezaminazzjoni se issir fil-klinika tal-podjatrija minn fejn ġejt reklutat/a u f' ħin konvenjenti għalik. Din il-laqgħa se tieħu madwar siegħa.

Waqt din il-laqgħa jiena nkun nista':

1. Nistaqsi xi mistoqsijiet dwarek, pereżempju l-età' tiegħek, l-piż, it-tul u xi mistoqsijiet dwar is-saħħa tiegħek.
2. Iż-żewġ saqajn se jigu eżaminati u bħal ma' intqal qabel diversi metodi tat-ttestjar mhux invażivi bħal '10g monofilament', '128Hz Tuning fork', 'Neurothesiometer', 'VibraTip' u 'NC-Stat DPN check' se jiġu użati għad-dijanjosi tan-newropatija periferali ikkawżata mid-dijabete.

M'intix obbligat/a li twieġeb il-mistoqsijiet kollha u il-parteċipazzjoni tiegħek f'dan l-istudju hija għażla għal kollox volontarja fejn tista' twaqqaf il- parteċipazzjoni fi xħin trid mingħajr ma tagħti l-ebda raġuni billi tinforma r-Riċerkatriċi jew is- Superviżura fuq d-dettalji ta' kuntatt li jinstabu fl-aħħar ta' din l-ittra. Jekk inti tixtieq twaqqaf il-parteċipazzjoni tiegħek, dan mhux ħa jkollu riperkussjonijiet negattivi fuqek u l-informazzjoni li tinġabar mingħandek titħassar u ma' tiġix użata. Nassigurak li se tinżamm il-kunfidenzjalità matul l-istudju kollu u l-identità tiegħek u kull informazzjoni personali miġbura mhuma se jiġu żvelati mkien fit-teżi, ir-rapporti, il-preżentazzjonijiet u/jew il-pubblikazzjonijiet li jistgħu jirriżultaw minnha. Kull tagħrif miġbur se jiġi psewdonomizzat, jiġifieri id-data kollha se tkun protetta permezz ta' sistema ta' kodiċi u miżmuma separatament mill-informazzjoni personali. Ir-Riċerkatriċi biss ser ikollha aċċess għall-informazzjoni miġbura, filwaqt li s-Superviżura akkademika u l-eżaminaturi se jkollhom biss aċċess għal data kkodifikata. Is-Superviżuri akkademiċi u l-eżaminaturi jista jkollhom bżonn aċċess għall-informazzjoni miġbura għal skop ta' verifika.

Id-data kollha se jinħażnu fuq il-kompjuter personali tar- Riċerkatriċi permezz ta' kodifikazzjoni tad-data (data encryption) u li hi protetta b'password. Barra minn hekk, il-materjal stampat se jinqafel f'post sigur.

F'każ li tħoss li l-istudju ħoloqlok diffikultà u tixtieq li tiddiskuti x'qed tħoss ma' professjonist/a mill-qasam tal-kura tas-saħħa, mill- 'Richmond Organization' se j/tkun qed j/tipprovdi servizz ta' għajnuna mingħajr ħlas min-naħa tiegħek. Tista' tikkuntatja lil 'Richmond Foundation' billi iċċempel fuq in-numru 21224580 jew tibat imejl fuq 'info@richmond.org.mt'.Jekk tiddeċiedi li tipparteċipa, tifhem li mhux se tirċievi ebda benefiċċju dirett u m'hemmx ebda effetti adversarji jew ebda riskju. Imma bil-parteċipazzjoni tiegħek f'dan l-istudju se tigi eżaminat/a fid-dettal għal newropatija periferali ikkawżata mid-dijabete.

Inti se tingħata kopja tal-ittra ta' informazzjoni u tal-formula ta' kunsens sabiex tkun tista' taċċessahom fil-futur. Barra minn hekk, skont ir-Regolamenti Ġenerali dwar il-Protezzjoni tad-Data (GDPR) u l-leġiżlazzjoni nazzjonali li timplimenta u tispeċifika aktar il-provvedimenti relevanti tar-regolamenti msemmija, inti għandek id-dritt li taċċessa, tirretifika, u fejn japplika titlob sabiex titħassar id-data li tikkonċerna lilek. L-informazzjoni personali kollha se titħassar hekk kif jintemm dan l-istudju ta' riċerka u jkunu ppubblikati r-riżultati miksuba.

Dan l-istudju ġie approvat mill-Kumitat għall-Etika fir-Riċerka fi ħdan il-Fakultà tax-Xjenzi tas-Saħħa fl-Università ta' Malta.

Grazzi ħafna tal-ħin u s-sehem tiegħek f'dan l-istudju. F'każ li jkollok xi mistoqsijiet jew tixtieq tiċċara xi ħaġa, tista' tikkuntatja lili (Riċerkatriċi) jew il- Superviżura tar-Riċerka fuq d-dettalji ta' kuntatt li jinstabu fl-aħħar ta' din l-ittra.

Dejjem tiegħek,

Ms. Stephanie Pleven
Riċerkatriċi

Prof. Cynthia Formosa
Superviżura tar-riċerka

Appendix 8- Consent Form in English

L-Università ta' Malta

<u>Participants` Consent Form</u>

'A Comparison of Screening Tools for the accurate diagnosis of Peripheral Neuropathy in Type 2 Diabetes'

I, the undersigned, give my consent to take part in the study conducted by Stephanie Pleven. The purpose of this document is to specify the terms of my participation in this research study.

1. I have been given written and verbal information about the purpose of the study and all questions have been answered.
2. I understand that I have been invited to participate in a study, in which the researcher will ask questions and perform tests is to compare and confirm the accurate diagnosis of diabetic peripheral neuropathy using different commonly used screening tools when compared to nerve conduction testing using NC-Stat DPN check.
3. I am aware that the meeting will take approximately 1 hour. I understand that data collection is to be conducted in the Podiatry Clinic where I was recruited from and at a time that is convenient for me.
4. I am aware that my responses and data will be written on the prepared record forms.
5. I am aware that the data collected will be coded and stored securely and separately from any personal data on the researcher`s personal password protected computer in an encrypted format. Any material in hard-copy form will be placed in a locked cupboard and kept until results are published.

6. I am aware that the researcher is the only person who has access to this data. The academic supervisor and examiners will typically have access to coded data only. There may be exceptional circumstances that allow the supervisor and examiners to have access to personal data too, for verification purposes.
7. I am aware that my identity and personal information will not be revealed in any publications, reports, or presentations arising from this research. All data collected will remain confidential and pseuronymised by storing in code.
8. I also understand that I am free to accept, refuse or stop participation at any time without giving any reason by contacting the Researcher or Supervisor on the contact details provided. This will have no negative repercussions on me and that any data collected from me will be erased and omitted from this study.
9. I also understand that my contribution will serve to help in contributing to a better understanding of which screening tool can reach an accurate and early diagnosis of Diabetic Peripheral Neuropathy.
10. If I feel distressed as a result of participation in this study Richmond Organization will be available to provide a service at no financial costs on my part. The Richmond Organization can be contacted by either phoning on 21224580 or via email on 'info@richmond.org.mt'. I understand that I will not be receiving any direct benefit and there are no known or anticipated risks of harm from participating in this study.
11. I understand that under the General Data Protection Regulation (GDPR) and national legislation that implements and further specifies the relevant provisions of said regulation, I have the right to access, rectify, and where applicable ask for the data concerning me to be erased.

12. I also understand that once the study is completed and results are published the data will be retained in an anonymous form. Any personal details will be destroyed.
13. I will be provided with a copy of the information letter and consent form for future reference.
14. I have read and understood the points and statements of this form. I have had all the questions answered to my satisfaction, and I agree to participate in this study.

Participant: ______________________________

Signature: ______________________________

Date: ______________________________

Ms. Stephanie Pleven
Researcher

Prof. Cynthia Formosa
Research Supervisor

Appendix 9- Consent Form in Maltese

L-Università ta' Malta

Formula ta' Kunsens tal-Parteċipanti

'A Comparison of Screening Tools for the accurate diagnosis of Peripheral Neuropathy in Type 2 Diabetes'

Jien, hawn taħt iffirmat/a, nagħti l-kunsens tiegħi biex nieħu sehem fl-istudju mmexxi minn Stephanie Pleven. L-għan ta' dan id-dokument hu li jiġu speċifikati t-termini tal-parteċipazjoni tiegħi f'dan l-istudju ta' riċerka.

1. Jien ingħatajt informazzjoni miktuba u verbali dwar l-għan tal-istudju u l-mistoqsijiet kollha twieġbu.
2. Nifhem li se nkun qed nipparteċipa fi studju, fejn ir- Riċerkatriċi ħa tikumpara u tikkonferma liema metodu tat-ttestjar għad-dijanjosi tan-newropatija periferali ikkawżata mid-dijabete jipprovdi dijanjosi aktar preċiża meta imqabbel mal- itesstjar tal-konduzzjoni tan-nervituri mill-użu ta' 'NC-Stat DPN check'.
3. Naf li l-istudju se jieħu madwar siegħa. Nifhem, l- ezaminazzjoni se issir fil-klinika tal-podjatrija minn fejn ġejt reklutat/a u f' ħin konvenjenti għalija.
4. Jien konxju/a li r-risposti tiegħi se jinkitbu r-risposti fuq formuli apposta.
5. Barra min hekk, naf li d-data se jinħażnu fuq il-kompjuter personali tar-Riċerkatriċi permezz ta' kodifikazzjoni tad-data (data encryption) li hi protetta b'password u din se tinżamm separatament mill-informazzjoni personali. Barra minn hekk, naf li l-materjal stampat se jitqiegħed f'post sikur u se jinżamm sakemm joħorġu r-riżultati.
6. Naf ukoll li r- Riċerkatriċi hi l-unika persuna li se jkollha aċċess għal din l-informazzjoni, filwaqt li s-Superviżura akkademika u l-eżaminaturi se jkollhom aċċess għal data

kkodifikata biss. Is-Superviżuri akkademiċi u l-eżaminaturi jista jkollhom bżonn aċċess għall-informazzjoni miġbura għal skop ta' verifika.

7. Naf li l-identità tiegħi u l-informazzjoni personali mhuma se jinkixfu mkien fit-teżi, fir-rapporti, fil-preżentazzjonijiet u/jew fil-pubblikazzjonijiet li jistgħu jirriżultaw minnha. Nifhem li se tinżamm il-kunfidenzjalità u kull data miġbura se tiġi psewdonomizzat, jiġifieri id-data kollha se tkun protetta permezz ta' sistema ta' kodiċi.

8. Nifhem ukoll li jien liberu/a li naċċetta, nirrifjuta jew inwaqqaf il-parteċipazzjoni f'kull ħin bla ma nagħti raġuni billi ninforma r-Riċerkatriċi jew is-Superviżura fuq d-dettalji ta' kuntatt li jinstabu fl-aħħar ta' din l-ittra. Dan mhux ħa jkollu riperkussjonijiet negattivi fuqi. Nifhem ukoll li la darba nirtira minn dan l-istudju, l-informazzjoni miġbura se titħassar u ma' tiġix użata.

9. Nifhem ukoll li l-kontribuzzjoni tiegħi ser isservi biex ikollna aktar għarfien dwar liema huwa l-aħjar metodu biex tilħaq dijanjosi preċiża u l-aktar kmieni ta' newropatija periferali fid- dijabete biex nipprevenu kumplikazzjonijit u ko-morbożitajiet futuri.

10. Madanakollu, jekk inħoss li l-istudju ħoloqli diffikultà u nixtieq li niddiskuti x'qed inħoss, naf li 'Richmond Organization' se j/tkun qed j/tipprovdi servizz ta' għajnuna mingħajr ħlas min-naħa tiegħi. Nista' nikkuntatja lil 'Richmond Foundation' billi nċempel fuq in-numru 21224580 jew nibat imejl fuq 'info@richmond.org.mt'. Jien nifhem li mhux se nirċievi ebda benefiċċju dirett u m'hemmx ebda effetti adversarji jew ebda riskju bil- parteċipazzjoni tiegħi.

11. Nifhem ukoll, li skont ir-Regolamenti Ġenerali dwar il-Protezzjoni tad-Data (GDPR) u l-leġiżlazzjoni nazzjonali li timplimenta u tispeċifika aktar il-provvedimenti relevanti tar-regolamenti msemmija, jiena għandi d-dritt li naċċessa, nirretifika, u fejn japplika nitlob sabiex titħassar id-data li tikkonċernani.

12. Naf ukoll li meta jintemm l-istudju u r-riżultati jkunu ppubblikati, l-informazzjoni personali miġbura titħassar.
13. Fl-aħħar nett, naf ukoll li se ningħata kopja tal-ittra ta' informazzjoni u tal-formula ta' kunsens sabiex inkun nista' naċċessahom fil-futur.
14. Jien qrajt u fhimt il-punti u d-dikjarazzjonijiet f'din il-formula. Inħossni sodisfatt/a bit-tweġibiet li ngħatajt għall-mistoqsijiet li kelli, u qed naċċetta minn jeddi li nippartecipa f'dan l-istudju.

Partecipant:______________________________

Firma:______________________________

Data:______________________________

Ms. Stephanie Pleven

Prof. Cynthia Formosa

Appendix 10- Data Collection Sheet

Participant Code Number:	
Demographic Data	
Age	
Gender	
Medical & Social History	
Body Mass Index	
Duration of Diabetes Mellitus	
Blood Glucose Level (mmol/L or mg/dL)	
Glycated Hemoglobin (HbA1c)	
Smoking Status	
List of Medication	
History of Surgical procedures	

10g Monofilament

Sensation Points of 10g monofilament	Left Foot	Right Foot
Present sensation		
Absent sensation		

128Hz Tuning Fork

Vibration Perception at the Hallux	Left Foot	Right Foot
Present sensation		
Absent sensation		

Neurothesiometer

Vibration Perception at the distal plantar aspect of hallux	Left Foot	Right Foot
Low Risk (0 to 20.99V)		
Medium Risk (21 to 30.99V)		
High Risk (≥ 31V)		

128-Hz ETF Descending Mode

Vibration Perception at the dorsal aspect hallux	Left foot	Right Foot
Low Risk (7 to 25 secs)		
Medium Risk (4 to 7 secs)		
High Risk (< 3 secs)		

128-Hz ETF Constant Mode

Vibration Perception at the dorsal aspect hallux	Left Foot	Right Foot
Present sensation		
Absent sensation		

NC-Stat® DPN Check®

Sural Nerve conduction Test (amplitude vs Conduction velocity)	Left foot	Right Foot
Non- Neuropathic		
Mild Neuropathy		
Moderate Neuropathy		
Severe Neuropathy		

Appendix 11- The results of the Kolmogorov-Smimov test & Additional Statistical Tests

i. Chapter 4.1- Kolmogrov-Smirnov test

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
128 Hz ETF Descending	.052	378	**.016**	.984	378	.000
Neurothesiometer	.138	378	**.000**	.883	378	.000

ii. Chapter 4.4- Chi-Square Test (P-value) – Comparing all Screening Modalities for present or absent sensation

Chi-Square Tests

	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	41.719[a]	5	.000
Likelihood Ratio	47.595	5	.000
Linear-by-Linear Association	31.299	1	.000
N of Valid Cases	756		

iii. Chapter 4.4- Cramer's V test- Comparing all Screening Modalities for present or absent sensation

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.235	.000
	Cramer's V	.235	.000
N of Valid Cases		756	

iv. Chapter 4.5.1- Chi-Square Test (P-value) – Relationship between the 10-g monofilament with NC-Stat® DPN Check®

Chi-Square Tests

	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	16.735[a]	2	**.000**
Likelihood Ratio	13.398	2	.001
Linear-by-Linear Association	6.680	1	.010
N of Valid Cases	126		

v. Chapter 4.5.1- Cramer's V test- Relationship between the 10-g monofilament with NC-Stat® DPN Check®

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.364	.000
	Cramer's V	**.364**	.000
N of Valid Cases		126	

vi. Chapter 4.5.2 - Chi-Square Test (P-value) – Relationship between the 128-Hz traditional tuning fork with NC-Stat® DPN Check®

Chi-Square Tests

	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	40.534[a]	2	.000
Likelihood Ratio	39.451	2	.000
Linear-by-Linear Association	37.041	1	.000
N of Valid Cases	126		

vii. Chapter 4.5.2 - Cramer's V test- Relationship between the 128-Hz traditional tuning fork with NC-Stat® DPN Check®

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.567	.000
	Cramer's V	.567	.000
N of Valid Cases		126	

viii. Chapter 4.5.3 - Chi-Square Test (P-value) – Relationship between the neurothesiometer with NC-Stat® DPN Check®

Chi-Square Tests

	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	28.698[a]	2	.000
Likelihood Ratio	27.142	2	.000
Linear-by-Linear Association	28.368	1	.000
N of Valid Cases	126		

ix. Chapter 4.5.3 - Cramer's V test- Relationship between the neurothesiometer with NC-Stat® DPN Check®

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.477	.000
	Cramer's V	.477	.000
N of Valid Cases		126	

x. Chapter 4.5.4 - Chi-Square Test (P-value) – Relationship between the 128-Hz ETF descending with the NC-Stat® DPN Check®

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	21.932[a]	4	.000
Likelihood Ratio	20.091	4	.000
Linear-by-Linear Association	12.853	1	.000
N of Valid Cases	126		

xi. Chapter 4.5.4 - Cramer's V test- Relationship between the 128- Hz ETF descending with NC-Stat® DPN Check®

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.417	.000
	Cramer's V	.295	.000
N of Valid Cases		126	

xii. Chapter 4.5.5 - Chi-Square Test (P-value) – Relationship between the 128-Hz ETF Constant with the NC-Stat® DPN Check®

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	25.334[a]	2	.000
Likelihood Ratio	24.192	2	.000
Linear-by-Linear Association	22.559	1	.000
N of Valid Cases	126		

xiii. Chapter 4.5.5 - Cramer's V test- Relationship between the 128- Hz ETF Constant with NC-Stat® DPN Check®

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	.448	.000
	Cramer's V	.448	.000
N of Valid Cases		126	