NEUROPROTECTION BY TOCOTRIENOLS IN TYPE 1 AND TYPE 2 DIABETES MELLITUS "VENUS"

Protocol no: VENUS Version 18

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Table of Contents

1. Introduction:

Diabetes Mellitus is a complex metabolic disease that can have devastating effects on multiple organs in the body. It has become a major public health problem affecting an increasing number of individuals worldwide. Diabetes is the leading cause of nephropathy, retinopathy, neuropathy and cardiovascular diseases. The characteristic clinical signs and symptoms of these complications are well established (American Diabetes Association, 2002; American Diabetes Association, 2008). The development of these complications is dependent on the duration of diabetes and the level of metabolic control including glucose level, hyperlipidemia, and other related parameters. Since both randomized trials and large cohort studies have shown that good control of blood glucose levels is associated with reduced risk of these complications (Gaede et al.,2003; Reichard and Rosenqvist, 1989; The DCCT Research Group, 1993), current treatment is aimed at obtaining and maintaining normal glucose levels.

Neuropathy affects approximately 30–50% of all diabetic patients and is the commonest form of neuropathy in the developed world. It encompasses several neuropathic syndromes including focal and symmetrical neuropathies. By far, the commonest of which is distal symmetrical neuropathy. The two main clinical consequences, foot ulcerations sometimes leading to amputation and pain neuropathy, are associated with much patient morbidity and mortality. There is now little doubt that glycaemic control and duration of diabetes are major determinants of distal symmetrical neuropathy. In addition, potentially modifiable, traditional markers of macrovascular disease such as hypertension, hyperlipidemia and smoking are also independent risk factors (Tesfaye, 2007).

There is now increasing evidence that the cause of distal symmetrical neuropathy may be nerve ischemia, though metabolic factors may be important early. Pain is the most distressing symptom of neuropathy and the main factor that prompts the patient to seek medical advice (Tesfaye, 2007). About 16-26% of diabetes patients experience chronic neuropathic pain (Jensen et al, 2006).

Abnormalities of autonomic function are very common in subjects with longstanding diabetes; however, clinically significant autonomic dysfunction is uncommon. Several organ systems including the cardiovascular, gastrointestinal and genitor-urinary systems may be affected (Tesfaye, 2007).

Oxidative stress is involved in the pathophysiology of diabetes mellitus and has a major role in the development of diabetic complications. This occur either because of free radical overproduction (by auto-oxidation of glucose and glycated protein) or by antioxidants level reduction (Young et al, 1992). The presence of free radical has an important role in nerve tissue damage that leads to diabetic neuropathy (Tutuncu at al,1998).

Electrophysiological studies have a major role in the measurement, detection, and characterization of peripheral neuropathy associated with diabetes (standardized measures in diabetic neuropathy (American Diabetes Association Consensus Statement, 1992)).

Vitamin E can be prescribed to patients with diabetes to prevent any oxidative damage (Srivastsan et al, 2009). Vitamin E is a powerful antioxidant that reduces levels of free radicals and oxidative stress.

An animal study have found that vitamins C and E treatment can lower malondialdehyde levels and increase the antioxidant levels to near control values. The results verify the presence of oxidative stress in diabetes and suggest beneficial effects of vitamins C and E combinations in combating the oxidative stress among diabetic rats (Aksoy et al, 2005).

Another animal study revealed that treating rats with α-tocopherol and tocotrienol for 10 weeks significantly improved and ameliorate all the biochemical and behavioral outcomes of alcohol-induced neuropathy in a dose-dependent manner with more potent effects observed with tocotrienols. The study demonstrates the effectiveness of tocotrienols in attenuation of alcoholic neuropathy (Tiwari et al, 2009).

In a placebo-controlled, double-blind, randomized study of 21 patients with type 2 diabetes, large doses of vitamin E were studied for their ability to reduce neuropathy. During the six-month study, patients were either given placebo or 900 mg vitamin E, then measured for nerve conduction and function. The researchers found that mild to moderate defective nerve conduction was improved with high-dose vitamin E, which suggested that patients with neuropathy might experience a reduction in symptoms with vitamin E treatment (Tutuncu et al, 1998).

Cognitive dysfunction is a less addressed and not as well recognized complication of diabetes. Patients with type 1 and type 2 diabetes mellitus have been found to have cognitive deficits that can be attributed to their disease. Both hypoglycemia and hyperglycemia have been considered as causes of cognitive dysfunction, and frequent recurrence of hypoglycemia will impair memory over time. Both old age and diabetes are independently associated with an increased risk of cognitive dysfunction; the risk is even greater for older adults with diabetes (Allen et al, 2004). Cognitive Function is the term used to describe a person's state of consciousness (alertness and orientation), memory, and attention span. Cognitive functioning has been the subject of many studies in both type 1 and type 2 diabetes. Several cross-sectional and case-control studies since 1980s have shown positive associations between diabetes and cognitive impairment (Gregg and Brown, 2003). Still, several questions remained to be answered. In type 2 diabetes, neuropsychological studies have reported moderate degrees of cognitive impairment. The most common findings are that diabetes is associated with lowered performance on speed of information processing test, episodic memory test, and to lesser extent, on mental flexibility test (Stewart and Liolitsa, 1999; Awad et al, 2004). Middle-aged individuals with type 1 diabetes have also been reported to show deficits on a wide range of neuropsychological tests compared to age-matched controls, but results are even more heterogeneous than in type 2 diabetes with respect to the severity and nature of the affected cognitive domains. Some studies reported impairments on tests relying on problem-solving skills (Deary et al, 1993), whereas other studies reported deficiencies in psychomotor efficiency (Ryan et al, 1992) or memory and learning (Ryan et al, 1993; Sachon et al, 1992) or found no difference at all (Wredling et al, 1990).

In addition, there is also evidence on the association of diabetes with changes in

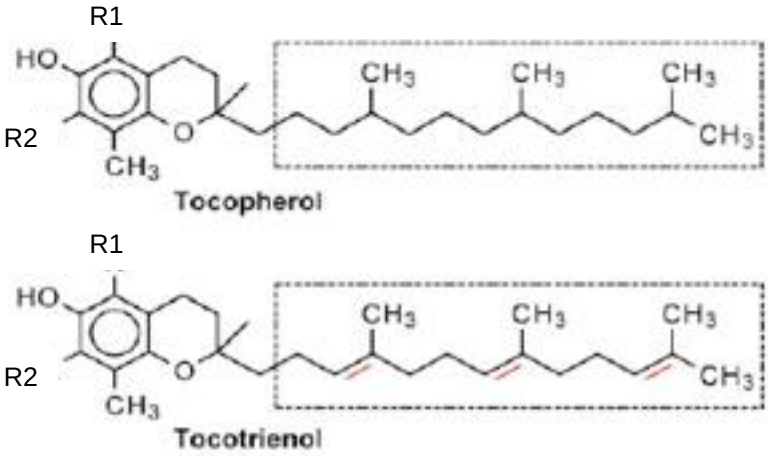
psychological performance. For example, it has been found that depressive symptoms are more prevalent in diabetic patients with type 1 or type 2 diabetes in comparison with age-matched controls (Anderson et al, 2001), and depressive symptoms might be attributed to cognitive dysfunction (Elderkin-Thompson et al, 2003; Lockwood et al, 2002).

Moreover, the structural correlates and pathophysiological mechanisms underlying these cognitive deficits are still uncertain. Kumar et al (2009) reviewed a few studies performed on type 1 and type 2 diabetic patients using magnetic resonance imaging (MRI). Subcortical white-matter and cortical and subcortical atrophy have been reported as radiological abnormalities. The limitation of majority of these studies involved small sample sizes or lacked appropriate non-diabetic controls. In type 2 diabetes, more studies have been published on MRI abnormalities, including cortical and subcortical atrophy, together with an increased occurrence of cerebral infarcts relative to controls. Moreover, white-matter lesions are more prevalent and more severe in type 2 diabetes in comparison with non-diabetic controls.

Atrophy may be linked to a history of severe hypoglycemic episodes, since only patients who had experienced multiple severe hypoglycemic episodes showed cortical atrophy (Perros and Frier, 1997). Focal lesions mostly involve the subcortical white- matter (Dejgaard et al, 1991; Ferguson et al, 2003a; Perros and Frier, 1997). For example, high-intensity periventricular white-matter lesions, particularly small punctuate white-matter lesions, periventricular caps, or pencil thin rims were present in one-third of the scans in a study by Ferguson and co-workers (Ferguson et al, 2003). They found these to be related to the presence of background retinopathy. It has been suggested that Vitamin E, including tocopherols and tocotrienols, can help to improve cognitive function and stall cognitive decline through its antioxidant effects. A reason for this nutrient's success at preventing oxidative damage in brain cells is its fat-soluble criteria (http://ageing.oupjournals.org). During the World Alzheimer's Congress held in July 2001, it was reported that high intakes of vitamin E effectively lessened memory loss and cognitive dysfunction among more than 6,000 elderly subjects who were generally taking Vitamin E between 200 to 400 IU per day (Kuhad et al, 2009).

1.2 Tocotrienols

The term vitamin E includes a group of plant derived lipid-soluble compounds. Their molecular structure is based on a chromanol ring with a side chain at the C2 position. In case of tocopherols, the phytyl side chain is saturated. In tocotrienols the side chain is unsaturated by the presence of three double bonds. There are 8 naturally occurring isoforms: 4 tocopherols and 4 tocotrienols, which are designated α-, β-, γ- and δ-, respectively, depending on the number and position of methyl groups at the chromanol ring (Figure 1).



Isoform	R1	R2
α-	CH3	CH3
β-	CH3	H
γ-	H	CH3
δ-	H	H

Figure 1: Chemical structures of Tocopherol and Tocotrienol. Note the three double bonds in the tocotrienol side tail. The number and position of methyl groups at the chromanol ring for corresponding isoforms is given in the table

The unsaturated side-chain in tocotrienols makes them penetrate tissues with saturated fatty layers more efficiently (Kuhad and Chopra, 2009).

Animal studies have also revealed that palm tocotrienols improved blood glucose, dyslipidemia and oxidative stress in diabetic rats. It is able to prevent the progression of vascular wall changes occurring in diabetes mellitus (Roper et al, 2000; Budin et al, 2009).

The oxygen consumption rate of the brain is high. Moreover, polyunsaturated fatty acids are found abundantly in the neuronal cell membranes. It has been hypothesized that cumulative free-radical damage to neurons over time contributes to cognitive decline and neurodegenerative diseases. Therefore, ingestion of sufficient supplemental antioxidants (such as tocotrienols) might provide some protection (Morris et al, 2002). This hypothesis was supported by the results of a clinical trial involving 341 patients with Alzheimer's disease of moderate severity who were randomly assigned to receive a placebo, vitamin E (2,000 IU/day dl-alpha-tocopherol), selegiline (a monoamine oxidase inhibitor), or vitamin E and selegiline together. After 2 years, vitamin E and selegiline, given alone or in combination significantly delayed brain functional deterioration (Morris et al, 2002).

Tocotrienols, in particular α-tocotrienol have been shown to possess neuroprotective effect independent of anti-oxidant activity (Sen et al, 2000; Khanna et al, 2005). Using cell-based studies, Sen et al (2000) have shown that α-tocotrienol but not α-tocopherol is able to prevent glutamate-induced neuronal cell death at nanomolar concentrations. Later studies conducted by Khanna et al (2005) showed that α- tocotrien ol conferred protection against glutamate and stroke-induced neurodegeneration in rats.

In view of the above neuroprotective property of tocotrienols, Kuhad and Chopra (2009) have proceeded to demonstrate that tocotrienols supplementation helped to reverse neuropathic pain in diabetic rats. It has been postulated the beneficial properties of tocotrienols are due to their suppressive effects on the oxidative-nitrosative stress, inflammatory cytokine release and caspase-3 which are implicated in the pathogenesis of diabetic neuropathy.

In the same year, Tiwari et al (2009) have shown that tocotrienols can prevent cognitive deficits and attenuate alcoholic peripheral neuropathy associated with selective neuronal damage due to chronic alcohol consumption. Moreover, the beneficial effects were found to be more pronounced with tocotrienols compared to tocopherols. It has been postulated that the anti-oxidants property of tocotrienols, the suppression of nitrosative

stress and elevated cytokines levels together with acetylcholinesterase activity in the brain regions contributes significantly in preventing the chronic alcohol- induced cognitive deficits in rats.

Yuen and his group are currently conducting a clinical study in human subjects on neuroprotective effects of tocotrienols (NCT00753532). In the study, subjects were followed up for 2 years to determine the volume of white matter lesions on repeated MRI after treatment with tocotrienol as compared to placebo. White matter lesions are related to vascular events in the brain and represent subclinical infarcts, resulting in death/ degeneration of neurons and are positively correlated to cognitive impairment. Preliminary results from an interim analysis are encouraging; patients on tocotrienols shown significant reduction in volume of white matter lesion (confidential communication).

Giving that the tocotrienols have been shown to possess neuroprotective effects and that both type 1 and type 2 diabetes can lead to peripheral neuropathy and cognitive impairment, the present study aims to determine the beneficial effects of tocotrienols in ameliorating such neurological related events in both type 1 and type 2 diabetic patients.

2. Aims and Objectives

Primary objective:

- To investigate the effects of tocotrienols on peripheral neuropathy in type 1 and type 2 diabetes mellitus.

Secondary objective:

- To investigate the effects of tocotrienols on cognitive impairment in type 1 and type 2 diabetes mellitus.
- To investigate the prevalence of cognitive impairment among diabetic patients.

3. Outcomes

Primary Outcomes

- Total Symptoms Score (TSS) (pain, paresthesia, burning, and numbness) of patients with diabetes peripheral neuropathy.

Secondary outcomes

- Neuropathy Impairment Score (NIS) of patients with diabetes type 1 and 2 neuropathy.
- Nerve Conduction Velocity (NCV) test of patients with diabetes type 1 and 2 neuropathy.
- Mini Mental State Examination (MMSE) score, Montreal Cognitive Assessment (MoCA) test.

4. Study Design

4.1 Study Population

Inclusion Criteria

- Diabetic adults (both type 1 or 2) ≥20 years old with diabetic peripheral neuropathy with TSS ≥ 3 points.
- Patients with type 1 diabetes (duration of ≥5 years).
- Patients with type 2 diabetes (at diagnosis).
- Patients with NIS ≥ 2

Exclusion Criteria

- Patients HbA1c >12%.
- Patients with hypoglycemia or conscious impairment at the time of test conduction.
- Patients exhibiting symptoms of peripheral vascular disease with absence of 2 foot pulses on the same foot (Posterior tibialis, Dorsalis pedis)
- Immuno-compromised patients.
- Patients with severe visual impairment, history of psychosis; schizophrenia; bipolar disorder; current depression or brain trauma and patients with alcohol dependence or drug abuse such as cocaine, heroin, etc.
- Those having lesions with a propensity to bleed (e.g., bleeding peptic ulcers), those having a history of hemorrhagic stroke and those with inherited bleeding disorders (e.g., hemophilia) or patients on warfarin.
- Pregnancy and lactation.
- Patients with renal function test of more than 150 umol/L (serum creatinine).
- Patients with liver function test of more than 5 times of the upper normal range (for AST, ALT and GGT)
- Active infection or infectious diseases.
- Other significant uncontrolled medical illnesses that may interfere with drug administration or interpretation of results.

4.2 Experimental Design

In this randomized, double-blind placebo-controlled study:

1. Three hundred patients with diabetes (type 1 and type 2) with diabetic peripheral neuropathy will be recruited from Seberang Jaya Hospital and 10 peripheral health clinics and hospitals, in Penang and Seberang Perai area.

2. The patients will be randomised to receive mixed tocotrienols (200 mg twice per day) or placebo for 12 months. Data on long term (up to 5 years) supplementation with 400mg/day of mixed tocotrienols did not show any reported or observed adverse effects (Nesaretnam et al, 2010; Magosso et al, 2010; and an ongoing clinical trial on the neuroprotective effects of tocotrienols. (http://clinicaltrials.gov/ct2/show/NCT00753532).

3. Patients with normal homocysteine level (less than 15 µmol/L) will be assigned as subgroup A. Patients who are found to have abnormally elevated homocysteine levels (equal or more than 15 µmol/L) will be assigned as subgroup H and they will be supplemented with Folic acid and methylcobalamin to treat the elevated homocysteine level.

4.At the screening stage, laboratory assessment for homocysteine, liver function test, kidney function test and HbA1c for each patient will be conducted.

5. At the baseline of the study (0 time), clinical assessment on clinical neuropathy, cognitive function will be assessed on the 300 patients. Other clinical laboratory tests include homocysteine level, tocotrienols level, folic acid, Vitamin B_{12}, Vitamin B1 and fasting blood glucose.

6. If the screening evaluation (clinical assessment, laboratory tests and questionnaires) shows that patients are eligible for the study, patients will be randomly assigned as Group 1 and Group 2. They will have 50/50 chance (like flipping a coin) of being in one of either group. One group will receive tocotrienols for one year as a supplement while the other group will be receiving placebo in addition to their normal anti-diabetic medications (in a double-blinded model). However, information regarding which treatment of each patient receiving will be made available to physician in case of any emergency.

7.Plasma tocotrienols will be assessed at the baseline, 3^{rd}, 6^{th}, 9^{th} and 12^{th} months.

8.The subjects are required to return for clinical assessment at 6^{th} and 12^{th} months interval starting from 0 time for one year. These assessments include clinical assessment of peripheral neuropathy (TSS and NIS) and cognitive function test using Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA). Laboratory assessment including fasting blood glucose, HbA1c, Liver function test, Renal function test and Tocotrienols plasma level will be assessed on the 3^{rd}, 6^{th}, 9^{th}, and 12^{th} month. Homocysteine, Folic acid, Vitamin B12 and Vitamin B1 will be assessed on baseline, 6^{th} and 12^{th} month.

9. Blood sample of 10 ml volume will be collected at baseline, 3^{rd}, 6^{th}, 9^{th} and 12^{th} month for the above mentioned laboratory tests.

10. Nerve conduction test (NCT) will also be performed on consented subjects at 0 time (baseline) and repeated only at 12^{th} month (end of study) to evaluate the conduction performance of the nerve fibers. Researchers will be blinded in the treatment given.

11. MRI will be done for 40 randomly selected (20 on tocotrienols and 20 on placebo) patients with at 0 time (baseline) and repeated only at 12^{th} month (end of study). MRI will also be done on randomly selected 20 non-diabetic individuals with no cognitive impairment as negative control at 0 time (baseline) only. The selection of volunteers will be made by the person in charge of the randomization. Researchers will be blinded in the treatment given.

12.Tocotrienol will be stored and dispensed in ready-packed bottle. Each bottle will contain 30 capsules. Tocotrienol/placebo will be given to the patients every 3 months during their visits to the research center.

13. Patients who successfully complete the initial 1-year course of the study will be invited to participate in an extended study where open label treatment will be provided for 1 year, to further observe the effect of the treatment in compliant patients, Procedures carried out during follow-up visits of the extended 1-year study will be similar to procedures in the initial study.

14. Withdrawal criteria:

Patient will be withdrawn from the study for any of the below reasons:

- Presence of adverse reactions whether it's related or not to this study.
- Patient shows poor compliance to tocotrienol/placebo. Researcher will ask patient to bring their bottle in the next visit. Researcher will count the remaining capsules to determine patient compliance.
- Patients withdraw their consent to participate in the study.

Tocovid SupraBio 200mg produced by Hovid Bhd (Ipoh, Malaysia) will be used in the study. This is the only 200 mg mixed tocotrienols product available commercially. Moreover, it is formulated with a patented delivery system that ensures consistent and enhanced oral absorption of tocotrienols. It is also used in the other studies mentioned above.

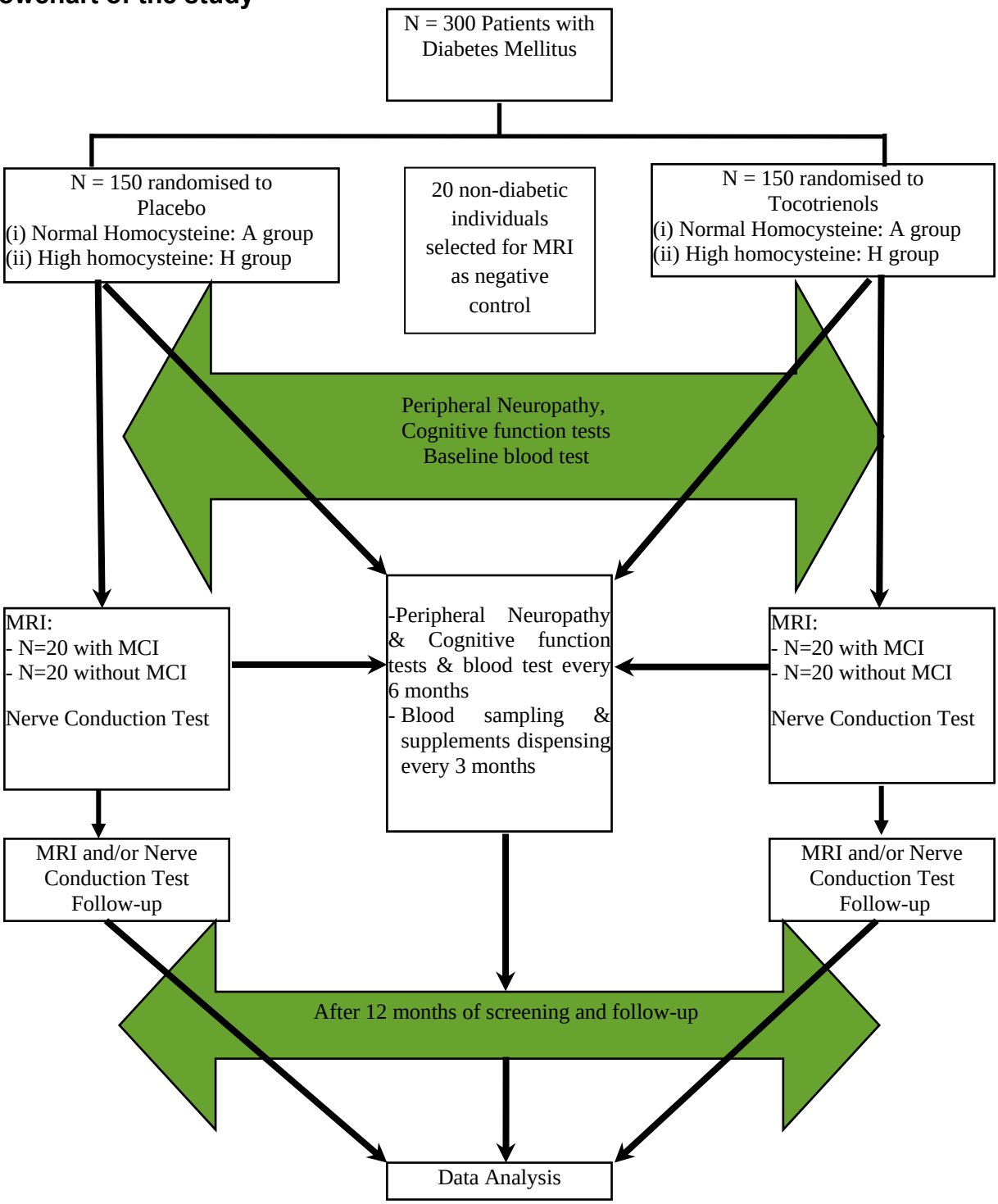
4.3 Study supplement

Tocotrienols is packed as 30 capsules per bottle, stored in amber color glass bottle and away from direct sun light. Bottles are stored in below 30°c according to the storage condition recommendations of the manufacturer.

4.4 Sample Size Calculation

We calculate the sample size for this study using PS: Power and Sample Size Calculation software version 3·1·2., based on the SYDNEY 2 trial which investigated the effects of alpha-lipoic acid on diabetic peripheral neuropathy (Ziegler *et al.*, 2006). We calculate the reduction in Total Symptom Score after treatment, with a corresponding standard deviation of 3·37. In order to achieve 90% power to detect a difference between groups of 1·33 points of the primary end point after 12-month of supplementation, a minimum of 136 subjects in each group are required. To accommodate a dropout rate of 10%, we increase the sample size to 150 per group, for a total of 300 participants.

457 **Flowchart of the study**



458

4.5 Measurements

4.5.1 Clinical assessment of peripheral neuropathy

In the present study, clinical assessment will be done through Total Symptoms Score (TSS). It is a questionnaire in which the patient is asked to assess the intensity (absent, mild, moderate, severe). Moreover, frequency (now and then, often, continuous) of four symptoms (pain, burning, paresthesia, numbness) is also assessed resulting in a scaled score in which 0 means no symptoms and 14.64 means that all four symptoms are severe and continuously happened. A change of 30% on this scale is considered to be clinically relevant (or ≥2 points in patients with a starting score ≤4 points) (Ziegler et al, 1995). This assessment will be conducted at the baseline of the study and every 6 months throughout the study period.

Neuropathy impairment score (NIS) is a summed score of neurological signs through which physicians will assess the patient. The NIS will be completed according to Dyck et al. (1997) at baseline, after 6 months, and 12 months for one year. A standard group of muscles will be evaluated for weakness and muscle stretch reflexes (biceps, triceps, brachioradialis, knee, ankle). Perceptions to touch pressure, vibration (128 Hz tuning fork), joint position, and pinprick perceptions will be graded on the index finger and the great toe as normal (0), decreased (1), or absent (2).

4.5.2 Nerve conduction test

Nerve conduction test will also be performed on consented subjects to evaluate the conduction performance of the nerve fibers. In the nerve conduction test, the sensory components of the following nerves will be assessed:

- Median nerves
- Radial nerves
- Sural nerves

The parameters assessed will be the conduction velocity, latency, and amplitude through the nerve. This assessment will be conducted at the baseline and 12^{th} month of the study starting from time “0”.

4.5.2 Blood parameters

Clinical laboratory tests will be conducted and the parameters investigated are:

- Fasting blood glucose
- HbA1c
- Liver function test
- Renal function test
- Homocysteine
- Tocotrienols plasma level
- Folic acid
- Vitamin B12
- Vitamin B1

Homocysteine, tocotrienol, folic acid, vitamin B_{12}, Vitamin B_1 and fasting blood glucose test will be conducted at the baseline of the study. Clinical laboratory test including fasting blood glucose, HbA1c, Liver function test, Renal function test and Tocotrienols plasma level will be assessed every 3 months throughout the 12 months study period (3^{rd}, 6^{th}, 9^{th}, and 12^{th} month). Homocysteine test will be used to diagnose vitamin B_{12} deficiency, and folate deficiency (Klee, 2000; Robertson et al, 2005; Pagana et al, 2010) Homocysteine, Folic acid, Vitamin B12 and Vitamin B1 will be assessed on baseline, 6^{th} and 12^{th} months.

4.5.4 Cognitive assessment tools

Cognitive tests that measure performance in specific domains of interest were chosen because they have been standardized, widely used, have well-established norms, and could be administered by non-neuropsychologists (Mungas et al, 2000). Because of the significant number of Malay-speaking participants, validated translations will be used for the adopted tests with the assistance of the School of Languages, Literacies and Translation, Universiti Sains Malaysia.

Patients will undergo cognitive dysfunction evaluation with the Mini Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA). These assessments will be conducted at the baseline of the study, at the 6^{th} and the 12^{th} month of the study period.

4.5.4.1 Mini Mental State Examination (MMSE)

Based on the 30-points questionnaires, the MMSE is a screening tool that is commonly used for recognizing alterations in cognitive function. It can also detect deficits in cognitive performance for older individuals without dementia. The range of scores is 0 to 30, with increasing scores indicating better cognitive function (Tombaugh and McIntyre, 1992). Cognitive impairment is indicated by this tool if the score is ≤ 24. The specificity of this tool is 96%, although, the sensitivity is weak (64%) (Lomholt and Jurgensen, 1998). It has been used in Malaysia to evaluate cognitive function among elderly pilgrims (Mimi, 2006).

4.5.4.2 Montreal Cognitive Assessment (MoCA)

The MoCA is a brief cognitive screening tool with high sensitivity and specificity to detect mild cognitive impairment (MCI) in patients performing within the normal range on the MMSE. It is a 10-minute cognitive screening tool designed to assist first-line physicians in detection of MCI, a clinical state that often progresses to dementia (Nasreddine et al, 2005; Zadikoff et al, 2008; Nazem et al, 2009). This tool is available in many languages to fit variant societies. In this study, a Malay version will be constructed and validated.

4.5.4.3 Data collection form

Demographic information and information pertaining to diabetes and its control will be collected from the data gathered during the clinic visit. These data included age, sex, type and duration of diabetes, HbA1c (A1C), Body mass index (BMI), kidney function, liver function, folic acid and B12 serum level, clinic or self-reported history of hypoglycemia, complications and the type of treatment for diabetes (insulin, oral, medications, and/or lifestyle modification).

4.5.5 Magnetic Resonance Imaging (MRI)

MRI will be conducted at baseline and at the end of the study to detect development/changes of lesions (WML) in the brain, changes in brain size and to measure the hemodynamic response (change in blood flow) related to neural activity in the brain or spinal cord of humans.

4.6 Ethical considerations

The study will be conducted after receiving approval from the ethics committee of the Ministry of Health Malaysia. Blood withdrawals are routinely performed on diabetic patients, thus do not represent a burden for the participants. MRI is a non invasive imaging technique that carries no significant risk for the participants. Nerve conduction test (NCT) has a very limited invasiveness and is routinely performed on diabetic patients with peripheral neuropathy. Cognitive function tests (MoCA & MMSE) are not invasive and are interview-based assessment tools.

4.6 Statistical Analyses

All the data collected along the trial will be compiled in Microsoft Excel sheet. Personal identifying information will be removed prior to statistical analyses except unique coding details, to allow data clarification if indicated. All data will be analyses according to intention-to-treat protocol.

Descriptive analyses will be performed for all continuous and categorical variables. All normally distributed continuous variables will be reported in mean (standard deviation), except otherwise specified. All categorical variables will be reported in frequency and proportion.

Inferential analyses will be performed for comparing variables between tocotrienols and placebo groups. Comparison for categorical variables between the two groups will be performed using Chi-square tests, or Fisher's exact test if the assumptions for Chi-square test were not met. Paired *t*-tests will be employed to detect differences all continuous clinical and biochemical markers before and after intervention in both groups at 6 and 12 months. Independent *t*-tests will be performed to compare these markers and adverse event rates across both groups. The two-sided statistical significance level, p-value, is set at 0.05 for all analyses in this study. All analyses will be conducted using Statistical Package for the Social Sciences (SPSS) version 23.0.

5. Research Gantt Chart

No.	Activities	2011	2012	2013	2014	2015	2016
1	**VENUS**						
i.	Patient recruitment	Oct/Nov			July		
ii.	Data collection					July	
iii.	Data analysis, final report					August	
iv.	Project completed					Nov	
2	**Extended study**						
i.	Patient recruitment					July	
ii.	Data collection						July
iii.	Data analysis, final report						August
iv.	Project completed						Nov

6. Significance of the study

1. Growing number of diabetic patients worldwide, including Malaysia.
2. Most diabetic patients suffer from neurodegeneration with no standard treatment.
3. Debilitation = high medical/social costs to the nation.
4. Tocotrienols, if proven effective, will have a big impact in the treatment of diabetes mellitus and the healthcare cost of the nation.
5. Tocotrienols are a truly Malaysian Product

7. Financial support

The study is supported by a research grant from the Malaysian Palm Oil Board (MPOB) and approved by PEMANDU.

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