Research Proposal

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1. Project Title

Elucidating the Effects of Tocotrienol rich Vitamin E in a Pre-diabetes Population of different ethnicities in Malaysia.

2. Project Synopsis

Diabetes is a major global health crisis that affects millions. In Malaysia, the rising prevalence of diabetes (i.e., from 11.6% in 2006, to 13.4 % in 2015 to 18.3 % in 2019) will be further aggravated by the conversion of pre-diabetes to diabetes, at the rate of 5-10% annually [1]. Notably, the prevalence of pre-diabetes in 2011 was reported as 22.1 % (30.2 % in men; 60.8% in women) [1]. A Finnish Study found a significant ($p=0.0004$) correlation between lower plasma α-tocopherol levels (i.e., 1 μmol/L decrease) with a higher prevalence of type 2 diabetes (22% increased diabetes risk) [2]. To date, there is no data available on vitamin E levels for both types of Vitamin E (Tocopherols and Tocotrienols) in Asian population. In addition, the role of Vitamin E in insulin resistance and beta cell function is not known.

T2DM is associated with central obesity, increase adipokines and insulin resistance. The peptide resistin confer insulin resistance, adiponectin increases its sensitivity while insulin action is marked by the increase in Glut 4 glucose transporter. Therefore, any changes in insulin resistance can be assess by measuring these peptides.

The primary aim of the study is to determine the levels of tocotrienol and tocopherol in pre-Diabetes population and to compare the Vitamin E isomers levels with normal and diabetes population. The second aim is to determine whether Tocotrienol-rich Vitamin E supplementation at 200mg per day given to pre-diabetes subjects will result in an improvement of insulin resistance and Beta cell function as assessed by the above peptides.

2.1 Problem Statement

The prevalence of Type 2 diabetes mellitus (T2DM) worldwide has rapidly increased; it is estimated that about 422 million adults were living with T2DM in 2014, compared to 108 million in 1980 [3]. In Malaysia, the prevalence of T2DM has shown an upward trend as reported by The National Health and Morbidity Surveys (NHMS) focusing on major ethnic groups; Malays, Chinese and Indians [1]. In 2015, the Malaysian National Health and Morbidity Survey IV (NHMSIV) reported the overall prevalence of T2DM (known and undiagnosed) among adults of 18 years and above was 17.5% (95% CI: 16.6, 18.3). The prevalence in Indians was the highest (22.6%) followed by Malays (14.6%); Chinese (12.0%) and other Bumiputra (2.0%) [1]. Recent reports by NHMS in 2019 showed an increased overall prevalence to 18.3% [4]. This ascending trend will be further aggravated by the conversion of pre-diabetes to diabetes, at the rate of 5-10% annually depending on population traits and pre-diabetes definitions [5].

Risk Factors: Although obesity and physical inactivity are known to be major risk factors for T2DM, oxidative stress could also contribute to the pathogenesis of T2DM by increasing insulin resistance or impairing insulin secretion [5,6,7]. A report by an Eastern Finnish study showed that a low α-tocopherol level is a risk factor of diabetes [2]. A pilot study by our group looking at levels of Vitamin E in the various ethnic populations in Johor found significantly lower levels of Tocopherols in the pre-diabetes subjects; with the Chinese having the highest levels and significantly higher than the Malays. At the same time, the levels of soluble receptors for advanced glycosylation end products (RAGE) were significantly higher in the diabetics suggesting an increase in oxidative stress caused by chronic hyperglycemia.

An important question to ask is whether a low vitamin E concentration is only for tocopherol or both. Whether these can precede the onset of diabetes, or the low concentration of vitamin E (both types) is a consequence of latent diabetes at the time of the baseline examination. To date, there is no data available on the different types of vitamin E levels in the Asian population. In addition, the role of Vitamin E in insulin resistance and beta-cell function is yet to be elucidated.

T2DM is associated with central obesity, increase adipokines and insulin resistance [7]. The peptide resistin confers insulin resistance [8], adiponectin increases its sensitivity [9] while insulin action is marked by the increase in Glut 4 glucose transporter. Therefore, any changes in insulin resistance can be assessed by measuring these peptides. T2DM also increase inflammation with consequence increase of inflammatory markers (IL-6, RAGE etc.).

Characterizing the effects of Vitamin E supplementation towards the development of T2DM between the local population will provide valuable information to the underlying mechanism towards disease susceptibility and gene mapping of complex diseases including Diabetes Mellitus.

Research Questions:

1. Will tocotrienol rich Vitamin E supplementation towards the Pre-Diabetes population helps to improved levels of insulin resistance and beta-cell function?

2. Will the supplementation of tocotrienol rich vitamin E on pre-diabetes subject affects the level of insulin, adiponectin, resistin, Glut 4 and what are the role of these peptides in PI3K/AKT and NF-κB pathway.

Study Objectives:

1) To determine the levels of tocotrienol and tocopherol in pre-Diabetes population and to compare the Vitamin E isomers levels with normal and diabetes population.
2) To determine whether Tocotrienol-Rich Vitamin E supplementation at 200mg per day given to pre-diabetes subjects will result in an improvement in the levels of Insulin, Insulin Resistance, Glut-4, Adiponectin and Resistin.
3) To determine the mechanisms of these effects through their role in PI3K/AKT and NF-*κ*B pathway.
4) To determine mechanistic effects of Tocotrienol-Rich Vitamin E in Pre-Diabetes of different ethnic groups by identifying differentially expressed proteins in Pre-Diabetes subjects with and without supplementation. (proteomics)
5) To elucidate the underlying mechanism of these differentially expressed protein through pathway analysis.

Hypothesis:

1) The levels of tocotrienol and tocopherol in pre-diabetes population is lower than in normal population and higher compared to diabetes population.
2) Supplementation of tocotrienol rich Vitamin E will reduce insulin resistance and increase the beta cell function.

3. Methodology

Part A: Cross Sectional Study

1) Target population and Sample collection.

The study involves the screening of subjects from the local population (Malay, Chinese, Indian and Orang Asli that shows the risk of development of diabetes mellitus/pre-diabetes following the FINDRISC Model. The Finnish Diabetes Risk Score (FINDRISC) is a diabetes risk score developed from the Finnish National Diabetes Programme (DEHKO 2001–2010) in a Finnish population. FINDRISC Model will be used as tools to screen samples and identify patients at risk of developing diabetes. This tool has been validated in multiple population. FINDRISC uses age, BMI, physical activity, vegetable & fruit intake, medical treatment of hypertension, history of hyperglycaemia and family history to determine risk of developing diabetes [10].

- A risk score of 0-14 points indicates a low to moderate risk of diabetes (1-17% chance of diabetes over 10 years).
- A risk score of 15-20 points indicates a high risk of diabetes (33% chance of diabetes over 10 years).
- A risk score of >20 points indicate a very high risk of diabetes (50% chance of diabetes over 10 years).
- Pre-Diabetes criteria: HbA1c: 6.0-6.5%

Comparisons of FINDRISC and current guidelines on screening for diabetes.

Table 4
Comparison of performance of FINDRISC and current guidelines on screening for diabetes.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's Index	Number eligible for screening (n, %)	Number of diabetes patients detected (n)
Undiagnosed diabetes							
ADA guideline	100	9.6	8.2	100	0.10	267 (91.1)	22
USPSTF	72.7	53.5	11.3	96.0	0.26	142 (48.5)	16
FINDRISC ≥12	81.8	58.3	13.7	97.5	0.40	131 (44.7)	18
FINDRISC ≥11	86.4	48.7	12.0	97.8	0.35	158 (53.9)	19

Recommendations for screening:
ADA: all adults 45 years of age and older; and those younger adults who are overweight or obese [BMI ≥ 25] and have at least one other risk factor, including physical inactivity, family history of diabetes, history of gestational diabetes or delivery of a baby weighing >4 kg, hypertension, HDL <0.9 mmol/L and/or triglyceride >2.8 mmol/L, women with polycystic ovarian syndrome, history of impaired fasting glucose or impaired glucose tolerance, other clinical conditions associated with insulin resistance (e.g., severe obesity), or history of cardiovascular disease (ADA 2009).
USPSTF: Adults aged 40–70 years who are overweight or obese.

Adapted from H.M. Lim, et al.,2020 'Performance of the Finnish Diabetes Risk Score (FINDRISC) and Modified Asian FINDRISC (ModAsian FINDRISC) for screening of undiagnosed type 2 diabetes mellitus and dysglycaemia in primary care. [11]. This present study by Lim et al also showed the performance of the FINDRISC in the screening of undiagnosed diabetes and dysglycaemia was good in a primary care setting in Malaysia. Further diagnostic blood testing for dysglycaemia is recommended in subjects with a FINDRISC score of ≥11 [11].

Ethical approval from Monash University Research and Ethics Committee (MUHREC) will be acquired. Written consent will be obtained from subjects aged 30 years-old and above, prior to recruitment.

Sample collection will be done at decentralized sample collection centres which are:

1. Thompson Hospital, Kota Damansara
2. Hospital UiTM, Sungai Buloh
3. CSJB area – Chinese and Orang Asli
4. Segamat area

Blood glucose level, HbA1c, lipid profile and levels of tocopherols and tocotrienols will be measured.

2) History-taking and Anthropometric Measurements

Demographic data will be gathered via interview questionnaire. Information such as age, gender, tribe, education, and occupation will be recorded. Health-related questions will include subject's past medical and surgical history, family history of cardiometabolic diseases and family/household/ contact history of infectious diseases, as well as subject's social history that included tobacco, alcohol, and recreational drug use. Complete history-taking and physical examination will be conducted during screening. Weight and height will be recorded twice without wearing shoes. Waist circumference will be measured by aligning the bottom edge of the measuring tape with the top of the hip bone and then, wrapping the tape measure all the way around the waist. The reading will be recorded twice during exhalation. The subject's blood pressure will be measured three times on the left arm in a sitting position, and an average of three stable readings will be taken.

3) Determination of Tocotrienols and tocopherol content using HPLC

The chromatographic system used will be an Agilent HPLC 1200 with fluorescence detector. Tocopherols and tocotrienols will be separated on a Phenomenex Kinetex^{TM} PFP column (5.0µm, 150 x 4.6 mm; Phenomenex) using methanol/H_2O (87:13) as an eluent at a flow rate of 0.9 mL/min. The fluorescence detector is set an excitation wavelength of 296 nm and emission wavelength of 325 nm and photomultiplier tube (PMT) gain at 6.

4) Blood Sampling and Biochemical Analysis

Venous blood sample from subjects will be collected to determine their blood glucose level, HbA1c, fasting lipids, levels of tocopherols, insulin, glucose, adiponectin, resistin, Glut-4,

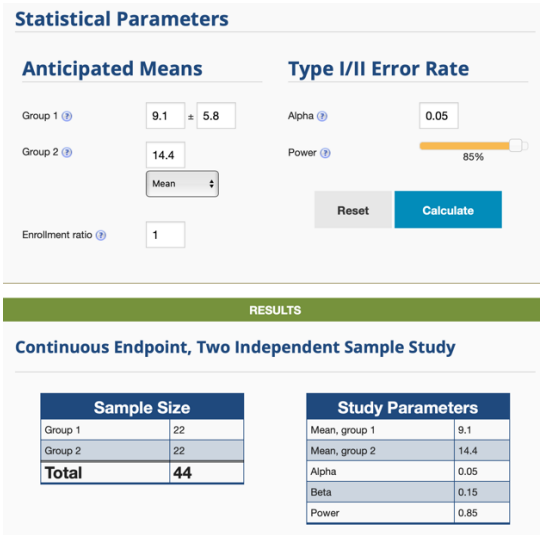
<u>Part B: Intervention of supplementation with 200mg daily of tocotrienol rich Vitamin E on Pre-Diabetes population for the duration of 3 month.</u>

1) Sample Selection.

The total sample size of the study is 240 participants. Each cohort (active vs placebo) comprises of 60 participants with an allowance of maximum 2 dropouts per cohort. This number is derived from an online sample size calculator available at: http://clincalc.com/stats/samplesize.aspx.

Malay n=60	Chinese n=60	Indian n=60	Orang Asli n=60
• 30 (Treatment) • 30 (Placebo)	• 30 (Treatment) • 30 (Placebo)	• 30 (Treatment) • 30 (Placebo)	• 30 (Treatment) • 30 (Placebo)

The data keys into the calculator for our study group design was “two independent study groups” with “continuous primary endpoint”. This implies that the two study groups will each receive different treatments and the primary endpoint is an average value of the data collected. For the statistical parameters, the anticipated means of fasting plasma insulin are based on a previous study by Kalman et al;2013 [12] with the value of 9.1 ± 5.8 for Group 1 (treatment) and Group 2 (placebo) was 14.4 ± 11.2. The enrolment ratio used was 1:1. The type I error rate (alpha cut-off) used was 5% (0.05) indicating a 5% chance of false positive. The type II error rate (beta cut-off) used was 20% (0.2) indicating a 20% chance of false negative. The results from the calculator are as shown below. Statistical analysis will be done using SPSS version 25.



5) Biomarkers Identification

ELISA will be carried out on selective Pre-diabetes samples that shows the effects of Vitamin E intervention i.e., improvement of insulin resistance levels, or beta cell function. Measurement of Insulin, Adiponectin, Resistin and Glut 4 will be done at baseline (0), 1st and 3rd months of the supplementation with 200mg daily of tocotrienol rich Vitamin E.

6) LC-MS (Proteomics)

Pre-Diabetes samples from each ethnic group will be selected for proteomics study.

7) Data Analysis

Data analysis will be done using SPSS version 26

Ethics Approval from:
Monash University Research and Ethics Committee (MUHREC)
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Target Population and Sample Collection

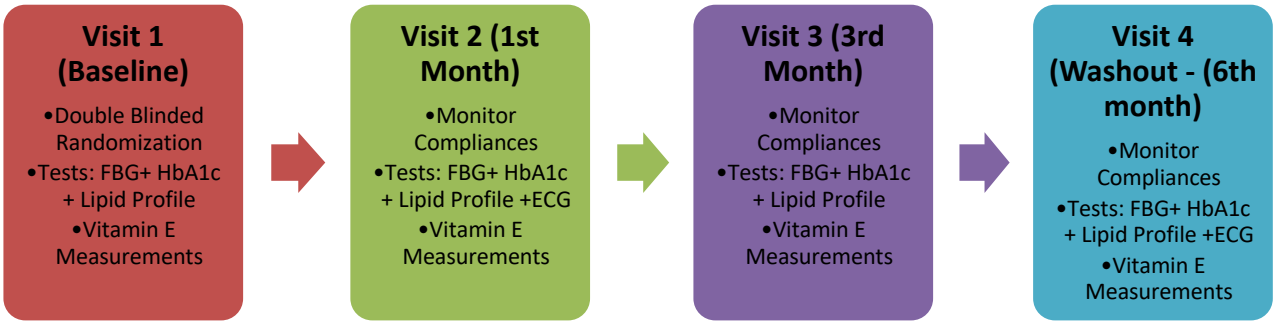
1) Screening:

- N=500
- The study subjects included a typical multi-ethnic population in Malaysia namely, Chinese, Malay , Indian and Orang Asli who are 40 years and above with risk of Diabetes Mellitus/Pre-diabetes.
- Follow the FINDRISC Model
- Pre-Diabetes criteria: HbA1c: 6.0-6.5%.
- Informed written consent will be distributed and explained to the participants prior to sample collection.
- Anthropometric measurements, blood pressure measurements, biochemical parameter analysis and health related questionnaire will be collected from participants.
- Measurement of Vitamin E (tocotrienols and tocopherols) level using HPLC
- Determination of blood glucose level, HbA1c, and lipid profile

Intervention of supplementation with 200mg daily of tocotrienol rich Vitamin E on Pre-Diabetes Population for the duration of 3 month.

1) Sample Selection:

- N=240
- Malay (Treatment=30, Placebo=30), Chinese (Treatment=30, Placebo=30), Indian (Treatment =30, Placebo=30) and Orang Asli (Treatment=30, Placebo=30).
- Measurement of Vitamin E (tocotrienols and tocopherols) level using HPLC.
- Determination of blood glucose level, HbA1c, and lipid profile.



Biomarker Measurements (ELISA)

- Insulin, Adiponectin, Resistin, Glut 4
- 3 timepoint. (Visit 1 (Baseline, Visit 2 (1^{st} month) , Visit 3 (3^{rd} month)
- N=120
- Malay (Treatment=15, Placebo=15), Chinese (Treatment=15, Placebo=15), Indian (Treatment =15, Placebo=15) and Orang Asli (Treatment=15, Placebo=15).

LC-MS (PROTEOMICS) and DATA ANALYSIS

PROJECT MILESTONES

TASKS / YEAR/MONTH	2021				2022				2023			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
ETHICS CLEARANCE AND PERMISSION FROM RELEVANT AUTHORITIES												
SCREENING AND PATIENTS' RECRUITMENT												
INTERVENTION STUDY: SUPPLEMENTATION OF TOCOTRIENOL RICH VITAMIN E												
DATA COLLECTION												
STATISTICAL ANALYSIS												
FINAL REPORT PREPARATION												
STUDY CLOSURE REPORT TO NIH/MOH: Study Findings Dissemination												

Q1: JANUARY – MARCH
Q2: APRIL-JUNE
Q3: JULY-SEPTEMBER
Q4: OCTOBER -DECEMBER

4. Expected Benefits from the Prediabetes Study

The expected outcomes from this project are several folds. ***Firstly***, it will address the research gap regarding the pathogenesis between Tocopherol and/or Tocotrienol deficiency with prediabetes. ***Secondly***, the study will determine the mechanisms of effects of Tocopherol and/or Tocotrienol on Insulin Resistance and prediabetes. *Thirdly*, it can potentially reduce the number of prediabetes converting to diabetes and developing macro- and microvascular complications. *Fourthly*, the study can potentially highlight and impart value-added products from the palm-oil industry into the medical sector, particularly for supplementation of prediabetes and as an adjuvant for diabetes patients to prevent or delay of diabetes complications. *Finally*, these benefits are consistent and align with some the sustainable development goals (SDG) such as good health and well-being (SDG #3), responsible consumption and production (SDG #12) and partnership for the goals (SDG #17).

5. Outcome measurements

Human Development – 1 HDR student (PhD)

Publication – 2 or 3 publications in high ranking or Q1 journals

Proposed/Potential Titles: -

- ✓ Tocotrienol Rich Vitamin E reduces insulin resistance and resistin and increase adiponectin in pre-diabetes and diabetes populations in Malaysia and reduces serum RAGE in newly diagnosed diabetes.
- ✓ The effects of Tocotrienol rich Vitamin E supplementation in pre-diabetes patients on T2DM biomarkers (resistin and adiponectin).

Potential Applications

- ✓ Tocotrienol rich Vitamin E supplementation in high-risk groups like prediabetes.

Tocotrienol rich Vitamin E as an adjuvant treatment to inflammatory disease states such as diabetes

- ✓ Tocotrienol rich Vitamin E as an adjuvant treatment to inflammatory disease states such as diabetes.

6. References

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