Study Protocol and Statistical Analysis Plan

June 30th 2023

The Effect of Vitamin D3 Therapy on 25(OH)D, 1,25(OH)2D, VDBP, and 24,25(OH)2D Maternal Serum Levels in Pregnant Women With Vitamin D Deficient and Insufficient

STUDY PROTOCOL

This is a randomized controlled trial of vitamin D therapy of 5,000 IU daily or 50,000 IU weekly during pregnancy. The study will be conducted at Cipto Mangunkusumo National Center General Hospital and Koja Distict Hospital in Jakarta, Indonesia from April 2021 – December 2023. All the pregnant women will be screened for eligibility when they present to the clinic for antenatal care visits and will be offered enrolment if they meet the following inclusion criteria: gestational age of ≤ 14 weeks, vitamin D deficient or insufficient (25(OH)D <30 ng/ml], and positive fetal heart rate from ultrasound examination. Individuals are not eligible if they have any of the following exclusion criteria: multiple pregnancy, pregnancy with congenital anomaly, hyperemesis gravidarum, diarrhea, complicated medical history (hypertension, diabetes mellitus, heart, kidney, or liver disease), or use of any dietary supplement containing vitamin D prior to enrolment. Informed consent will be obtained from all participants. The study was approved by ethical committee at Cipto Mangunkusumo National Center General Hospital (KET – 785/UN2.F1/ETIK/ PPM.00.02/ 2019).

Participants are randomly assigned to one of two parallel intervention groups, with allocation concealment: vitamin D3 (cholecalciferol) 5,000 IU/week (Imedco) or 50,000 IU/week (Imedco). Assignment is based on a computer-generated randomization list, with 1:1 allocation, using permuted blocks of size 6. All participants are given a standard prenatal multivitamin (Emineton) which contains fumarate Fe 90 mg; folic acid 0.4 mg; vitamin B6 3 mg; vitamin B12 5 mcg; sulphate cupric 0.35 mg, sulphate cobalt 0.15 mg, sulphate mangan 5 mcg, vitamin C 60 mg,

vitamin E 5 mg, and phosphate calcium 60 mg. Interventions in both groups are given for four weeks.

Baseline blood tests, including serum 25(OH)D, $1,25(OH)_2D$, VDBP, and $24,25(OH)_2D$ are performed at recruitment. A medical history, physical, and ultrasound examination are performed. Participants complete a questionnaire about sunlight exposure. Thereafter, participants are contacted weekly at their homes to evaluate the adherence and the symptoms related to pregnancy complications and hypo-/hypercalcemia. Adherence to the supplementation regimen is assessed by maternal self-report and capsule counts at the end of the study visit. After four weeks of interventions, the maternal venous blood is collected to assess serum 25(OH)D, $1,25(OH)_2D$, VDBP, and $24,25(OH)_2D$. Safety thresholds for the study include hypervitaminosis D (25(OH)D D ≥100 ng/mL).

Serum 25(OH)D is quantified by direct competitive Chemilumiscence Immunoassay (CLIA) using LIAISON®. Serum $1,25(OH)_2D$ is quantified by Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) using ImmuTube®. Serum VDBP is quantified by Enzyme-Linked Immunosorbent Assay (ELISA) using Quantikine®. Serum $24,25(OH)_2D$ is quantified by Acquity I Class Binary Solvent Manager FTN , Xevo TQXS Tandem Mass Spectrometry (Waters Corporation). All laboratory test will be performed at the Prodia Laboratory, Jakarta.

STATISTICAL ANALYSIS PLAN

Data will be processed using IBM SPSS version 25 program. Each dependent variable is tested for homogeneity variance using the Kolmogorov Smirnov normality test. Baseline data are described and summarized either by means and standard deviations for the normally distributed variables, or medians and interquartile ranges for the non-normally distributed variables. Bivariate analysis of qualitative data is tested using Chi-Square for the normally distributed variables or Fisher test for the non-normally distributed variables. Bivariate analysis of quantitative data is tested using T-test for the normally distributed variables or Mann Whitney U-test test for the non-normally distributed variables. The data is supplemented with 95% confidence interval with a significance limit of $p \leq 0.05$.