Investigating the effect of maternal vitamin D levels on infant vitamin D levels and determining the cut off values for hypovitaminosis D among pregnant mothers in Colombo District

By

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Thesis submitted to the University of Sri Jayewardenepura for the award of the degree of Doctor of Philosophy in Biochemistry

DECLARATION

The work described in this thesis was carried out by me under the supervision of Prof. G.S.H Liyanage, Prof. U.P.K Hettiaratchi, Prof. Shamini Prathapan, Dr. K.C.D.P. Silva and Prof. D.P.S. Gunasekara and the report on this has not been submitted in whole or in part to any university or any other institution for anther Degree / Diploma".

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Anusha Kaneshapillai Date

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LIST OF ABBREVIATION

MMN	Multiple Micro Nutrient
CSTH	Colombo South Teaching Hospital
OFD	Occipito-Frontal Diameter
APDAF	Anterio-Posterior Diameter of the Anterior Fontanel
TDAF	Transverse Diameter of the Anterior Fontanel
FFQ	Food Frequency Questionnaire
IFCT	Indian Food Composition Table
WHO	World Health Organization
rpm	Rotate per minute
PTH	Parathyroid Hormone
IP	Inorganic Phosphorous
ALP	Alkaline phosphatase
SD	Standard Deviation
ELFA	Enzyme Linked Fluorescent Assay
SPR	The Solid Phase Receptacle
ELISA	Enzyme Linked Immunosorbent Assay
HRP	Horseradish peroxidase
TMB	Tetramethylbenzidine
BCG	Bromocresol green
ERC	Ethics Review Committee
SPSS	Statistical Package for Social Sciences

IQR	Inter Quartile Range
VDD	Vitamin D Deficiency
VDI	Vitamin D Insufficiency
MOH	Medical Officer of Health
CMC	Colombo Municipality area
RDHS	Regional Director of Health Services
BSA	Body Surface Area
SOS	Speed of Sound
BMD	Bone Mineral Density
EDTA	Ethylenediaminetetraacetic acid
CLIA	Chemiluminescent Immuno Assay
RLU	Relative Light Units
ROC	Receiver Operating Characteristic
AUC	Area Under the Curve
D_2	Ergocalciferol
D_3	Cholecalciferol
25(OH)D	Calcidiol
$1,25(OH)_2$	Calcitriol
VDR	Vitamin D Receptors
IOM	Institute of Medicine
RIA	Radio Immuno Assay
HPLC	High Performance Liquid Chromatography

LC-MS/MS	Liquid Chromatography-tandem Mass Spectrometry
DEQAS	International vitamin external quality assurance scheme
p	Significance level
r	Correlation Coefficient
RR	Relative risk
CI	Confidence Interval
B	Unstandardized Coefficients

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ABSTRACT

Many studies from South Asian countries have reported vitamin D deficiency among many age groups. However, there is very little information on vitamin D levels in the Sri Lankan community. State sector clinics do not provide vitamin D supplementation to pregnant or lactating mothers. Whereas most mothers who are followed up at private sector receive vitamin D. Further, suitability of current cut off values for our population has not been explored. Hence, objectives of this study were to evaluate the effects of maternal vitamin D levels on infant vitamin D levels & to determine cut off values for hypovitaminosis D among pregnant mothers in Colombo District. Current study was carried in two phases. In first phase, a cohort of 105 pregnant mothers in 3rd trimester, from an Obstetric unit of Colombo South Teaching Hospital in Sri Lanka was recruited & followed up till 6 weeks post-partum to study the effect of maternal vitamin D status on infant vitamin D levels. In second phase, 393 pregnant mothers in 3rd trimester were recruited from Medical Officer of Health (MOH) areas in Colombo District to determine cut off values for hypovitaminosis D. An interviewer administered questionnaire was used to collect socio demographic, medical, dietary and lifestyle data. Venous blood sample was collected for analysis of vitamin D, parathyroid hormone (PTH), inorganic phosphorous (IP), calcium and alkaline phosphatase (ALP). Bone mineral density (BMD) was also measured in 2nd phase in addition to previously mentioned parameters. In phase one study, correlations and relative risks with 95% CI (RR) were

performed to find out associations. Receiver Operating Characteristic (ROC) curve was performed to find out possible cut off for hypovitaminosis D based on the PTH values as per the manufacturers cut off (66.5pg/mL). In phase one, mean vitamin D levels (ng/mL) in pregnant mothers, lactating mothers, infants were 18.6±7.2, 20.6±6.9 & 11.4±5.6 respectively. Vitamin D had significant negative correlation with PTH [pregnant mothers (r=-0.295;p=0.002), lactating mothers (r=-0.249;p=0.011) & infants (r=-0.280;p=0.004)]. Maternal vitamin D levels during both pregnancy (adjusted odds ratio/OR:1.151;95%CI: 1.030-1.288) & lactation (OR:1.237;95%CI;1.063-1.440) had a significant positive correlation with infant vitamin D levels. Mothers who fulfilled recommended daily dietary vitamin D (≥600 IU/day) had higher serum vitamin D levels than mothers who did not meet recommended dietary vitamin D levels (<600 IU/ day). Maternal vitamin D during 3rd trimester of pregnancy or infant vitamin D level had no significant association (p>0.05) with infant anthropometry. In phase two, the mean vitamin D level of pregnant mothers was 18.6±7.5 ng/mL. Similar to phase one study, PTH correlated well (r=-0.220; p=0.000) with serum vitamin D. In mothers, vitamin D levels significantly correlated with exposure to sunlight (r=0.315; p=0.000). However, BMD measures didn't show a significant correlation (p>0.005) with vitamin D levels. Cut off for hypovitaminosis D derived from this study was 8.1 ng/mL using ROC curves (lower than the Institute of Medicine/IOM cut off) with an accuracy rate of 95.6% (corresponding sensitivity & specificity were 0.961 & 0.667 respectively). Prevalence of hypovitaminosis D according to newer cut off is 8.6% (n=09), 1.9% (n=02) & 54.3% (n=57) in pregnant mothers, lactating mothers & infants respectively, when newer cut off value is applied for phase one sample. Prevalence of hypovitaminosis D according to newer cut off among pregnant mothers in the Colombo District is 4.1% (n=16).