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ORIGINAL ARTICLE

Placental Vitamin D Metabolism as Well as its Relations to Circulating Vitamin D Metabolites in Pregnancies

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ABSTRACT

Background: Uncertainty exists about the impact of placental vit D metabolism on mother's blood levels of vit D in people.
Objective: Awareness of placental vit D metabolism and how it affects the amount of vit D metabolites in mother's blood was one of the goals of this investigation.
Methods: To take part in a 14-week controlled feeding experiment, 27 third-trimester singleton pregnant women in good health from Hayatabad Medical Complex during Jan 2022 to April 2022. Female trophoblasts in vitro were further treated using Vitamin D3-13C to investigate the synthesis of vit D intermediates.
Results: In maternal tissue, there was a substantial connection between 24,25-Dihydroxycholecalciferol and 25-hydroxyvitamin D. These placental metabolites were also closely connected to the corresponding compounds found in maternal circulation. 3-epi-25-Hydroxyvitamin D3 and low density lipoprotein-related protein 2 with 25-hydroxyvitamin D; CYP2R1 with 3-epi-25-Hydroxyvitamin D3; Cubilin (CUBN) with 25-hydroxyvitamin D; CYP27B1 with 3-epi-25-Hydroxyvitamin D3 and 1,25-dihydroxyvitamin D and CYP24A1 with 25-hydroxyvitamin D, 24,25-Dihydroxycholecalciferol all showed positive relationships.
Conclusion: Strong relationships between the levels of circulating vit D metabolites in pregnancy and a number of placental markers of vit D metabolism suggest the hypothesis that the profile of vit D metabolites in mother's blood is altered by the placenta.
Keywords: 24,25-Dihydroxycholecalciferol (24,25(OH)2D3), 25-hydroxyvitamin D (25(OH)D3), 3-epi-25-Hydroxyvitamin D3 (epi-25(OH)D3), CYP2R1, CYP24A1

INTRODUCTION

Mother vitamin D (Vit D) or physiologically significant metabolites 25-hydroxyvitamin D and/or 1,25-Dihydroxyvitamin D should be delivered via the placenta because the newborn cannot synthesize vit D [1]. It is necessary to comprehend the regulatory mechanisms and rate-limiting processes of maternal vit D transport in order to choose prospective alternatives for focused intervention to improve maternal health [2].

The levels of vit D metabolites and related proteins in the blood have been found to fluctuate significantly throughout pregnancy in humans [3]. One such example is the 1.5–2.0-fold increase in circulation stages of 1,25-Dihydroxyvitamin D3, a functional vit D metabolite and a transmitter for circulating vit D metabolites [4]. Accordingly, elevations in 25-hydroxyvitamin D3, which is the main source of vit D in human blood and 25(OH)D_3 have also been found in a number of studies. However, it is still unknown what causes these trimester changes in the circulation of vit D metabolites [5].

The placenta is a prenatal organ that includes all of the enzymes involved in the metabolic mechanism for converting vit D in to its effective forms, including LDL related protein 2 (LRP2), Cubilin (CUBN) receptors, 1a-hydroxylase (CYP27B1), 25-hydroxylase (CYP2R1), and 24-hydroxylase (CYP24A1). As a result, it has the potential to influence the reported variations in maternal circulating vit D metabolites [6]. In fact, 1,25-dihydroxyvitamin D3 and 24,25-Dihydroxycholecalciferol ,are formed in the uterus through the activities of CYP27B1 gene and CYP24A1 gene on 25-hydroxyvitamin D in vitro [7]. The placenta's ability to control 1,25-dihydroxyvitamin D3 and 24,25-Dihydroxycholecalciferol levels in people is unknown [8]. Additionally, it is unknown if 25-hydroxyvitamin D3 is formed by placental CYP2R1 from cholecalciferol and whether the mother's blood may be exposed to this metabolite [9].

Identifying placental vit D metabolism and its impact on maternal circulation vit D metabolites in people was the objective of this research.

MATERIAL METHODS

A 14-week controlled feed intake experiment with 27 third-trimester singleton pregnant women (gestational age 25–28 weeks, >22 years old) was conducted in Hayatabad Medical Complex between Jan 2022 to April 2022. Participants received a mean daily intake of 510 IU of vit D, including 210 IU from just a prenatal multivitamin supplement and 310 IU from food, and were assigned randomly to 1 of 2 choline consumption levels. Each workday, pregnant women took one study meal and supplements. For analyses of vit D genomic and metabolic factors, during delivery, placental tissue samples were obtained, and blood from veins was taken on 1st week and 12th week (representing the research start and ending point). Due to the fact that three of the mothers gave a home birth and their placental tissue really wasn't available, 24 pregnant females were involved in the research.

Analysis of Vit D Metabolites In The Placenta And Blood: A stable isotope dilution Liquid Chromatography with tandem mass spectrometry technique was used to measure the amounts of plasma 24,25-Dihydroxycholecalciferol, serum 25-hydroxyvitamin D, and serum 3-epi-25-Hydroxyvitamin D3 [10]. After solid-phase extraction using Oasis HLB 6cc cartridges, liquid-liquid extraction adding 0.8 milliliter acetonitrile, 2000 µL MTBE, and 0.02 mL, and 0.2 mg/mL DMEQ-TAD derivatization, maternal 24,25-Dihydroxycholecalciferol and 25-hydroxyvitamin D3 was isolated of homogenized 0.2 g uterine tissues [11]. An international standard solution comprising 1.6 µmol/L of d6-24,25-Dihydroxycholecalciferol and 205 µmol/mL of d3-25-hydroxyvitamin D3 was added after the tissues had been homogenized. The isolates were washed in 120 µL of an 80:60 water/methanol solution to determine the amounts of vit D metabolites. [12].

Genotyping and MRNA Quantification of Vit D Metabolic Pathway and Elements in Uterine tissues: Single nucleotide polymorphism genotypes within the CYP2R1 and DBP, which are involved vit D metabolism, were identified. The RNeasy Microarray Tissue Mini Kit was applied to extract Ribonucleic acid (RNA) from samples of placental tissue, and NanoDrop equipment was used to measure the quantities. For reverse transcription cDNA Reverse Transcription kit was utilized, and an Applied Biosystems quantitative real-time PCR machine was used for the process.

Unidentified Vit D treatment and Real-Time Quantitative Reverse Transcription PCR of vit D metabolism enzymes: To determine how vit D medication affects the vit D enzymes' ability to express their genes in the womb, HTR-8/SVneo cells were used to

cultvate with unmarked type of Vitamin D3 and 25-hydroxyvitamin D3 for 72 hours. RNA was isolated, measured, and collected by the cell pellets of each sample, followed by quantitative real-time PCR and reverse transcription methods. The identical reaction conditions, data expression technique for CYP27B1 and CYP24A1 were utilized in quantitative real-time PCR process used for the uterine tissues.

Statistical Analysis: STATA version 14, JMP Pro version 12, and SigmaPlot version 11 software were all used to conduct the analyses. Arithmetic means, standard deviations (SD), or geometrical means were used to represent distributed data that are not normally arranged. The P value smaller than 0.05 was consider important. Using the Wilcoxon test, epi-25(OH)D_3 was the only exception, pregnant women's differences in the levels of the circulating vit D metabolites in the research were evaluated using t – test.

RESULTS

Vit D derivatives in the bloodstream and placentas of pregnant women: Characteristics and measurements: The demographic features and prenatal outcomes of 3rd-trimester pregnant women are shown in Table 1, and their blood levels of vit D metabolites at the beginning and end of the trial are shown in Table 2. For 25-hydroxyvitamin D3 and 3-epi-25-Hydroxyvitamin D3 variations were found between the start of study and conclusion; however, for 24,25-Dihydroxycholecalciferol and 1,25-dihydroxyvitamin D3 no variations were found between these study time periods.

Table 1: Basic traits and gestational outcomes in 3rd-trimester pregnant women

Characteristics	women in pregnancy (n = 24)
Age (Years)	25±4
Prepregnancy Body mass index (BMI)	26±3
Parity	
Primiparas	13
Multiparas	11
Prior usage of vitamin supplements	
Yes	21
No	3
Period of study	
May to October	11
November to April	13
GC and GT polymorphism	
GT	10
GG	8
GC	6
G<A polymorphism (CYP2R1)	
AG	3
AG	19
GC	2
G<A polymorphism (CYP2R1)	
AG	6
GG	16
AA	2
duration of pregnancy, week	38.4±1.8
Pregnancy weight increase	14.8±3.7
Mode of Delivery	
Vaginal and Cesarean	17+7

Table 2: Concentrations of circulating vit D metabolites in third-trimester pregnant women

Circulating vit D metabolites	Units	Start of research (n = 24)	End of research (n = 24)
25-hydroxyvitamin D3 or 25(OH)D_3,	nmol/L	84.3±27.6	95.8±32.0
1,25-dihydroxyvitamin D3 or 1,25(OH)$_2D_3$	Pmol/L	291 (236, 358)	314 (257, 378)
24,25-Dihydroxycholecalciferol or 24,25(OH)$_2D_3$	nmol/L	12.4±6.2	12.9±5.3
Free 25-hydroxyvitamin D3 or 25(OH)D_3	Pmol/L	15.8 (11.9, 20.8)	19.5 (15.1, 25.2)
3-epi-25-Hydroxyvitamin D3 or 3-epi-25(OH)D_3	nmol/L	3.3±2.3	4.6±3.6
24,25-Dihydroxycholecalciferol or 24,25(OH)$_2D_3$	nmol/L	0.14±0.05	0.14±0.04
DBP	µg/mL	462±248	423±232

Relationship between uterine and circulating vit D metabolites: Significant correlations (r = 0.83, P <0.001) over time were found between the majority of maternal circulating vit D and uterine vit D metabolites, namely 24,25-Dihydroxycholecalciferol and 25-hydroxyvitamin D3 (figure 1). At the beginning and conclusion of the trial, circulating 24,25-Dihydroxycholecalciferol and 25-hydroxyvitamin D3 revealed significant associations with placental 25-hydroxyvitamin D3 shown in Figure 1A and 1B. Along with starting point of research 24,25-Dihydroxycholecalciferol: 25-hydroxyvitamin D3 proportions, and end of research 3-epi-25-Hydroxyvitamin D3 and 25-hydroxyvitamin D3 (placental) showed a positive association with all of these variables (Figure 1C, 1D and 1E). Additionally, circulating 24,25-Dihydroxycholecalciferol placental metabolite demonstrated meaningful relationships with circulating 25-hydroxyvitamin D3 and 24,25-Dihydroxycholecalciferol (start of research) and with 3-epi-25-Hydroxyvitamin D3 (end of research) (Figure 1F, 1G and 1H).

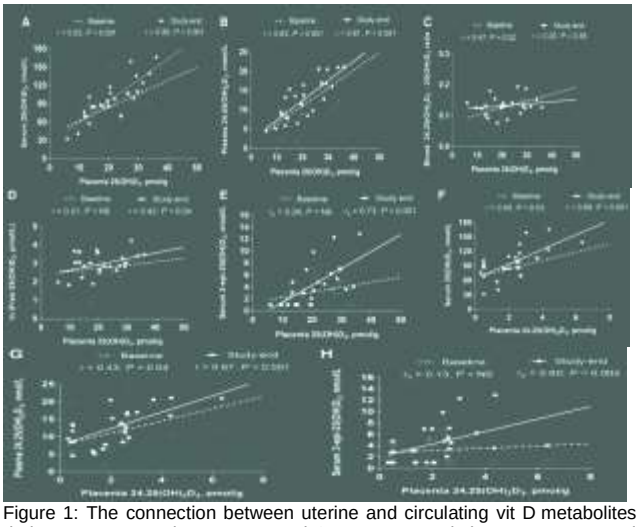

Figure 1: The connection between uterine and circulating vit D metabolites during pregnancy. The Pearson and Spearman correlation tests were used to derive the data.

Relationships between circulating vit D metabolites and the elements of the vit D biosynthetic pathway demonstrated genetically in the pregnancy: At start and end of research, circulating 25-hydroxyvitamin D3 levels, and concentrations of 3-epi-25-Hydroxyvitamin D3 and 25-hydroxyvitamin D3, were all positively correlated with placental LRP2 mRNA (Table 3). Additionally, there was a potential for starting circulation 3-epi-25-Hydroxyvitamin D3 level to correspond with parental LRP2 messenger RNA abundance. Gestational CYP2R1 messenger RNA availability was significantly linked with starting 3-epi-25-Hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 level, while gestational Cubilin (CUBN) messneger RNA plenty was consistently linked with circulating 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 concentration equally. When comparing baseline 3-epi-25-Hydroxyvitamin D3 and end of research 1,25-dihydroxyvitamin D3 concentrations, placental CYP27B1 mRNA abundance was favorably correlated. Additionally, circulating 1,25-dihydroxyvitamin D3 and initial concentrations of and 3-epi-25-Hydroxyvitamin D3, free 25-hydroxyvitamin D3 and 25-hydroxyvitamin D3 were all favorably correlated with placental CYP24A1 mRNA abundance.

Effects of vitamin D therapy on the control of uterine genes using HTR-8/SVneo growing cells: We looked at the results of treating unmarked cholecalciferol with vit D, 25-hydroxyvitamin D3, and 1,25-dihydroxyvitamin D3 affected the response of the

placental cells' gene expression to vit D metabolic enzymes. When compared to the ethanol control at 72 hours, the 1,25-dihydroxyvitamin D3 and 25-hydroxyvitamin D3 therapies significantly improved the expression of the CYP24A1 gene (Figure 2). Similar to this, the higher vitamin D3 treatment dosages led to an increase in CYP24A1 mRNA abundance that was dose-responsive (Table 4).

Table 3: Pregnant women's blood levels of the elements of the vit D metabolic pathway are associated with the quantity of their placental gene transcripts.

Embryonic genes	Circulating Vit D metabolites	r^2	(95 Percent CI)	P
LRP2	starting point			
	25-hydroxyvitamin D3	1.52	3.2 (1.8, 5.1)	<0.001
	Completion of research			
	25-hydroxyvitamin D3	1.58	1.8 (0.73, 3.1)	0.002
	25-hydroxyvitamin D3	1.33	8.6 (2.3, 15.1)	0.010
	3-epi-25-Hydroxyvitamin D3	1.64	22 (11, 37)	0.001
CUBN	starting point			
	25-hydroxyvitamin D3	1.26	0.013 (0.0022, 0.027)	0.022
	1,25-dihydroxyvitamin D3	1.48	0.0036 (0.0016, 0.0053)	0.002
	Completion of research			
	25-hydroxyvitamin D3	1.38	0.017 (0.0044, 0.029)	0.001
	1,25-dihydroxyvitamin D3	1.48	0.0033 (0.0016, 0.0053)	0.001
CYP2R1	starting point			
	1,25-dihydroxyvitamin D3	1.33	0.0024 (0.0008, 0.0041)	0.003
	3-epi-25-Hydroxyvitamin D3	0.54	1.22 (1.12, 1.33)	<0.002
	Completion of research			
	1,25-dihydroxyvitamin D3	1.33	1.22 (1.08, 1.033)	1.07
CYP27B1	starting point			
	3-epi-25-Hydroxyvitamin D3	1.41	1.18 (1.78, 1.32)	0.003
	Completion of research			
	1,25-dihydroxyvitamin D3	1.29	1.13 (4.5×10^{-6}, 1.27)	0.044
CYP24A1	starting point			
	25-hydroxyvitamin D3	1.44	1.25 (1.11, 1.38)	0.002
	1,25-dihydroxyvitamin D3	1.41	1.42 (1.18, 1.66)	0.002
	25-hydroxyvitamin D3	1.32	1.77 (1.23, 1.12)	0.006
	3-epi-25-Hydroxyvitamin D3	1.55	1.37 (1.23, 1.55)	<0.002
	Completion of research			
	1,25-dihydroxyvitamin D3	1.46	1.39 (1.19, 1.57)	0.003

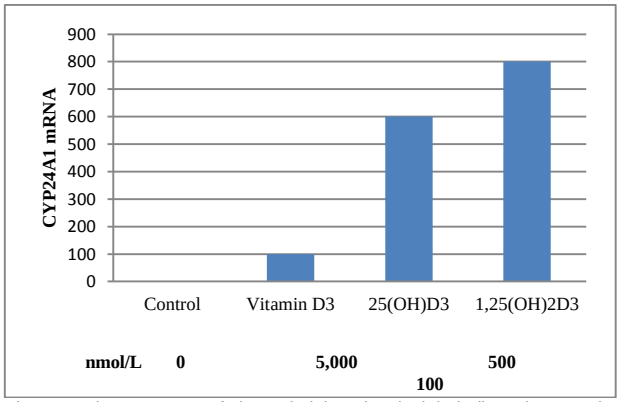

Figure 2: The outcomes of vit D administration (unlabeled) on the quantity of CYP24A1 mRNA in HTR-8/SVneo cells at 72 hours

Table 4: HTR8/SVneo cell responses to vit D (unlabeled) therapy at 72 hours in terms of CYP27B1 and CYP2R1 mRNA abundance

Treatment	CYP27B1	CYP2R1
Control	2.5 ± 0.5	1.5 ± 0.1
Vitamin D3 5,000 nM	3.2 ± 0.1	1.2 ± 0.2
25-hydroxyvitamin D3 500 nM	2.5 ± 0.3	1.3 ± 0.1
1,25-dihydroxyvitamin D3 100 nM	4.5 ± 0.2	1.2 ± 0.1

DISCUSSION

Due to the dearth of human research utilizing placental measures of vit D biomarkers, the modulatory consequences of the uterus on mother circulation vit D metabolites are unclear. In order to establish the connections between mother vit D metabolites, uterine gene regulation of vit D metabolic elements, and their interactions with circulating vit D metabolites, we examined samples taken from pregnant women during the 3rd trimester. Additionally, a study was done in vitro to examine how human placental trophoblasts metabolize and secrete a ^{13}Ccholecalciferol tracer.

Several beneficial correlations among maternal and circulating vit D compounds were discovered in this study, which examined uterine tissues and blood vit D compounds shown in Figure 1. Additionally, extra significantly, there were helpful correlations among the quantity of vit D metabolic elements in placental tissue and levels of circulating vit D metabolites, that were unaffected by the time of year, BMI, genetic variations linked to vit D, and the outcomes of gestation and neonatal development (Table 3). Overall, our findings support the idea that the placenta could influence the amounts of vit D compounds in the maternal circulation in expectant women.

Fascinatingly, vit D that is absorbed by the uterus appears to be $25(OH)D_3$ that is DBP-bound as opposed to free. For instance, the connection between placental and serum concentration i.e. $25(OH)D_3$ (which includes both free and DBP-bound forms) was greater at the beginning and conclusion of the trial than it was between free serum and placental i.e. $25(OH)D_3$. This result is in line with a previous research by Tamblyn et al. (2017), which found that serum bound to DBP and placental correlated more strongly than serum bound to free DBP. Additionally, during the third trimester, there was a encouraging relationship involving the serum total 25-hydroxyvitamin D3 concentration and the profusion of low density lipoprotein-related protein 2 and Cubilin mRNA in the placenta [13]. The amount of placental CUBN mRNA, on the other hand, did not show any correlations with free serum 25-hydroxyvitamin D3. When considered collectively, these results point to a preferential absorption of 25-hydroxyvitamin D3 bound to DBP by the uterus through a controlled mechanism involving the LRP2-CUBN receptors.

The positive linear connection between circulating 1,25-dihydroxyvitamin D3 and 25-hydroxyvitamin D3 concentrations in eight pregnant women that we previously found is consistent with a recent sizable randomized controlled trial investigation by Hollis et al., (2011) [14]. Therefore, it has been proposed that kidney or placental CYP27B1 produces 1,25-dihydroxyvitamin D3 during this reproductive stage in a substrate dependent manner i.e., 25-hydroxyvitamin D3. The circulating start point 25-hydroxyvitamin D3 concentration and research end point 1,25-dihydroxyvitamin D3 concentrations were favorably correlated with placental CYP27B1 mRNA abundance in the current study, which included the same group of eight expecting females. These results are consistent with the idea that maternal CYP27B1 adds to maternal blood circulation. The results of earlier in vitro studies that confirmed the generation and release of 1,25-dihydroxyvitamin D3 in the placenta are strongly supported by the human data from pregnant women.

This study's cell culture component demonstrates that maternal germ cells in lab culture are able of producing and secretion of 25-hydroxyvitamin D3 with the aid of vitamin D3. Despite the fact that Rubin et al., (1993) study noted that placental mitochondria produced 25-hydroxyvitamin D3, our finding that trophoblasts produce and transfer 25-hydroxyvitamin D3 implies that the pregnancy may be an extramedullary source of the

substance circulating in mother's blood. [15]. We further showed that CYP24A1 is over expressed in HTR-8/SVneo cells exposure to a variety of vit D3 dosages in a dose-dependent manner. This in vitro result points to a potential functional significance of the earliest form of vit D in the embryo by suggesting that, vit D3 can be regulated by target genes in female maternal trophoblasts.

CONCLUSION

A variety of connections between uterine and maternal vit D indicators from this research trial support the idea that the regulation of circulatory vitamin D compounds is actively influenced by pregnancy. Given that this study's cell culture component demonstrated that human trophoblasts may manufacture and produce 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 when provided cholecalciferol, it is feasible that the placenta might be used as a source of these vit D metabolites in human pregnancy.

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