

PREGNANCY

First trimester serum levels of 25-hydroxyvitamin D, free β -human chorionic gonadotropin, and pregnancy-associated plasma protein A in Spanish womenANA M. FERNÁNDEZ-ALONSO¹, CARLOS J. VALDERA-SIMBRÓN¹, GABRIEL FIOL-RUIZ¹, FIRMA RODRÍGUEZ-SÁNCHEZ², PETER CHEDRAUI³, & FAUSTINO R. PÉREZ-LÓPEZ⁴

The Spanish Vitamin D and Women's Health Research Group: ¹Department of Obstetrics and Gynecology, Hospital Torrecárdenas, Almería, Spain, ²Clinical Laboratory, Hospital Torrecárdenas, Almería, Spain, ³High Risk Pregnancy Unit, Enrique C. Sotomayor Obstetrics and Gynecology Hospital, Facultad de Medicina, Universidad Católica de Santiago de Guayaquil, Ecuador, and ⁴Department of Obstetrics and Gynecology, Hospital Clínico de Zaragoza, Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain

(Received 20 December 2010; revised 1 March 2011; accepted 3 March 2011)

Abstract

Background. Vitamin D has been implicated in embryo/placental development and growth; however information in this regard is limited or unavailable.

Objective. To assess 25-hydroxyvitamin D (25(OH)D), free β -human chorionic gonadotropin (β -hCG) and pregnancy associated plasma protein A (PAPP-A) status during pregnancy.

Methods. Serum 25(OH)D, β -hCG, and PAPP-A levels were measured in the first trimester of otherwise healthy Spanish pregnant women ($n = 488$). Rho Spearman coefficients were calculated to determine correlations between analytes.

Results. Median serum 25(OH)D levels for the entire sample was 27.4 ng/ml (interquartile range = 12.1). 25(OH)D levels were insufficient (20–29.99 ng/ml) and deficient (< 20 ng/ml) in 40.6% and 23.2%, respectively, in relation to ethnics, body mass index values, tobacco use, and season/gestational age at blood sampling. β -hCG and PAPP-A levels significantly correlated ($r^2 = 0.47$) yet neither of them with 25(OH)D levels. Despite this, the three analytes significantly correlated with gestational age at sampling.

Conclusion. First trimester 25(OH)D, β -hCG, and PAPP-A levels increase with gestational age; however, placental peptides do not correlate with vitamin D levels, suggesting a non-placental 25(OH)D production. More research is required in this regard.

Keywords: Vitamin D, 25-hydroxyvitamin D, β -hCG, PAPP-A, first trimester pregnancy

Introduction

Vitamin D precursors produced by skin photosynthesis and acquired by digestive absorption are transformed in the liver into 25-hydroxyvitamin D [25(OH)D] or calcidiol. In the kidneys and other tissues, a second hydroxylation produces the bioactive hormone $1\alpha,25$ -dihydroxyvitamin D [$1,25$ (OH)₂D] or calcitriol [1–3]. $1,25$ (OH)₂D has effects on bone metabolism and mineral homeostasis. During early pregnancy, vitamin D seems to be involved in implantation and placental development, displaying in addition antiproliferative and immunomodulatory actions [4–6]. Moreover, the human placenta has the enzymatic activity of 25-hydroxyvitamin D₃- 1α -hydroxylase (CYP27B1) and $1,25$ (OH)₂D₃-24-hydroxylase (CYP24A1), which are involved in vitamin D synthesis and metabolism [7]. *In vitro* studies, on the other hand, suggest that vitamin D may stimulate estrogen, progesterone, and beta human chorionic gonadotropin (β -hCG) synthesis [7,8] whereas inhibit cytokine secretion [9]. The aim of the present study was to measure first trimester serum β -hCG, pregnancy associated plasma protein A (PAPP-A) and 25(OH)D levels in Spanish women and determine analyte correlations.

Methods*Study design and participants*

This cross-sectional study was approved by the Research Ethics Committee of the Torrecárdenas Hospital, Almería, Spain. Pregnant women attending the outpatient clinic of the mentioned Hospital from May 2009 to April 2010 for their first antenatal visit (11–14 weeks gestation) were informed about the research, its objectives and requested to participate after giving written consent. All participants lived in the Spanish Mediterranean coast (near Almería at latitude 36° N, longitude 2° W). This geographical area has one of the sunniest, warmest, and driest climates in Europe. Indeed, it has nearly 3000 h of sunshine per year, 320 sunny days, and only 25–30 wet days [10]. Subjects with increased risk for intrauterine fetal growth restriction were excluded namely hereditary (factor V Leyden mutation, S protein deficiency, C protein deficiency, factor XII deficiency, methylenetetrahydrofolate reductase mutation) or acquired thrombophilias (antiphospholipid syndrome or systemic lupus erythematosus). Upon recruitment no participant was on vitamin D supplementation. Maternal data for analysis included: age, parity, season, and

gestational age at blood sampling, body mass index (BMI), ethnics (Caucasian yes/no), smoking status, residency (coast/highland), and nationality.

Serum 25(OH)D, β -hCG and PAPP-A assays

Serum 25(OH)D levels were determined by electrochemiluminescence immunoassay using a polyclonal antibody against 25(OH)D on a Roche Modular E 170 analyzer (Roche Diagnostics, West Sussex, England) [11]. Results are expressed in ng/ml (equivalence of 1 ng/ml = 2.496 nmol/l). This method has been validated against HPLC and radioimmunoassay methodology and is capable of detecting 25(OH)D levels in the range of 4–96 ng/ml [12]. Women were categorized according to determined 25(OH)D levels as sufficient (≥ 30 ng/ml), insufficient (20–29.99 ng/ml), and deficient (< 20 ng/ml).

β -hCG was measured by a solid-phase, two-site sequential chemiluminescent immunometric assay (Immulite 2000 Beta HCG, Siemens Healthcare Diagnostics Products Ltd) [12]. Results are expressed in ng/ml (equivalence of 1 ng/ml of free β -hCG = 1 mIU/ml of free β -hCG in terms of the World Health Organization's First International Reference Preparation of chorionic gonadotropin beta subunit human, number 75/551). Analytical sensitivity was 1 ng/ml.

PAPP-A was measured by a solid-phase enzyme-labeled chemiluminiscent immunometric assay [12]. Results are expressed in mIU/ml and analytical sensitivity was 0.025 mIU/ml.

Statistical analysis

Statistical analysis was performed using SPSS software package (Version 10.0 for Windows, SPSS Inc, Chicago, IL). Data are presented as medians, interquartile ranges (IQR), and percentages. The Kolmogorov–Smirnov's test was used to determine the normality of data distribution. According to this, continuous non-parametric data were analyzed with the Mann–Whitney test (two independent samples) or the Kruskal–Wallis test (various independent samples). Rho Spearman coefficients were calculated to determine correlations between 25(OH)D, PAPP-A, and β -hCG levels. Prior to any linear regression analysis, numeric data were log-transformed. A *p* value of < 0.05 was considered as statistically significant.

Results

During the study period a total of 507 gravids (11–14 weeks) were recruited. Four hundred eighty-eight participants providing serum in adequate conditions for 25(OH)D, PAPP-A, and β -hCG level determinations. Basal characteristics and 25(OH)D, β -hCG, PAPP-A status of the studied population are depicted in Table I. Of the total, 406 were Spanish and 82 were immigrants. Median serum 25(OH)D levels for the entire sample was 27.4 ng/ml (IQR = 12.1). Only 36.3% of subjects had adequate serum 25(OH)D levels whereas in 40.6% and 23.2% these levels were found to be insufficient and deficient, respectively. Lower levels were significantly related to ethnics (non-Caucasian), nationality, BMI values, season at blood sampling and tobacco use (Table I). Women sampled at higher gestational age displayed significantly higher 25(OH)D, β -hCG, and PAPP-A levels.

β -hCG and PAPP-A levels did not statistically differ in accordance to 25(OH)D status (Table II). β -hCG and PAPP-A levels significantly correlated (r^2 : 0.47, $p = 0.0001$)

yet neither of them with 25(OH)D levels. Despite this, all analytes significantly correlated with gestational age at sampling.

Discussion

The human placenta is a particular endocrine organ, source of many proteins which are pivotal for obstetrical outcome [13–16]. This organ is also responsible for inflammatory and immune changes that protect fetal growth and development [17,18]. Placental peptide (i.e. β -hCG and PAPP-A) and fetal nuchal translucency measuring have been used to screen several fetal malformations, trisomies and adverse pregnancy outcomes [19,20]. hCG is produced by the syncytiotrophoblast and its main role is to maintain the corpus luteum and progesterone secretion during the first trimester of pregnancy [13–15]. PAPP-A is used in prenatal genetic screening and as a marker of atherosclerosis [19–21]. During pregnancy, low PAPP-A levels have related to a higher risk for intrauterine fetal growth restriction, trisomy 21, preterm birth, preeclampsia, placental abruption, and stillbirth [19,22].

Despite its metabolic importance, the vitamin D endocrine system also participates in different reproductive-related processes both in females and males, including among others sperm quality, implantation, cell proliferation, local immune response, and placental hormone/cytokine secretion [1,3–9,23–25]. Serum 25(OH)D is a marker of endogenous vitamin D status with a half-life of approximately 3 weeks. Cholecalciferol has a short half-life (approximately 24 h); therefore its serum levels depend on recent sunlight exposure and vitamin D intake. 1,25(OH)₂D is the bioactive endocrine compound, with a shorter half life (4–6 h) and a potency some 1000 times higher than its precursor. Eighty percent of 25(OH)D is bound to its binding protein, 15% to albumin and 0.03% circulates freely for metabolic transformation [1–3,26,27]. Low serum 25(OH)D levels have been associated to several obstetrical complications [1,3,28]. Although criteria for adequate serum vitamin D level definition are still a subject of debate, reports indicate that at least 30 ng/ml are required to maintain general health [1,2,29]. In the present series, a high rate of women presented 25(OH)D levels below this cut-off value. Factors predicting these levels and their impact on obstetrical outcome will be the matter of discussion in future publications.

The present analysis found that first trimester 25(OH)D, β -hCG and PAPP-A levels positively correlated with gestational age at sampling. Although β -hCG and PAPP-A displayed a positive correlation, neither analytes correlated with vitamin D, suggesting that 25(OH)D first trimester increase in relation to gestational age is not of placental origin. Clinical data supporting our findings are lacking in the literature. Reports indicate that β -hCG and PAPP-A levels correlate [30] and steadily increasing in the first trimester of pregnancy [31]. Our data seem to support these facts; however, to best of our knowledge correlation with vitamin D status has not been reported in the clinical scenario.

1,25(OH)₂D enhances *in vitro* hCG secretion (a marker of trophoblast endocrine activity) and regulates vitamin D hydroxylase gene expression in human syncytiotrophoblast cells [7]. Indeed, during pregnancy, 1 α -hydroxylase increases at the placental (and macrophages) level incrementing therefore 1,25(OH)₂D levels [32]. Our results seem to suggest that maternal 25(OH)D serum levels are capable of providing the required amount of the active hormone 1,25(OH)₂D in order to maintain placental β -hCG and PAPP-A production. In one *in vitro* study, a vitamin D

Table I. Basal characteristics and 25 (OH) D, β -hCG, PAPP-A status of the studied population ($n = 488$).

Parameters	25 (OH) D levels (ng/ml), median [IQR]	β -hCG levels (ng/ml), median [IQR]	PAPP-A levels (mIU/ml), median [IQR]
All ($n = 488$)	27.4 [12.1]	1.32 [1.10]	0.60 [0.51]
Age			
< 20 ($n = 27$)	28.8 [11.9]	1.32 [1.35]	0.71 [0.74]
20–29 ($n = 214$)	26.1 [13.7]	1.33 [1.15]	0.58 [0.46]
≥ 30 ($n = 247$)	28.0 [11.0]	1.31 [1.07]	0.61 [0.52]
	$p = 0.14$	$p = 0.78$	$p = 0.60$
Caucasian			
Yes ($n = 431$)	28.1 [10.9]	1.31 [1.11]	0.60 [0.48]
No ($n = 57$)	16.0 [8.9]	1.40 [1.08]	0.76 [0.53]
	$p = 0.0001^*$	$p = 0.37$	$p = 0.05$
Nationality			
Spanish ($n = 406$)	27.8 [11.4]	1.32 [1.14]	0.60 [0.48]
Immigrants ($n = 82$)	23.8 [12.7]	1.30 [0.94]	0.68 [0.69]
	$p = 0.04^*$	$p = 0.66$	$p = 0.28$
Body mass index (kg/m ²)			
< 25 ($n = 294$)	28.3 [11.2]	1.32 [1.10]	0.58 [0.58]
25–29.99 ($n = 130$)	25.9 [12.5]	1.37 [1.16]	0.61 [0.50]
≥ 30 ($n = 64$)	23.7 [14.5]	1.14 [0.87]	0.64 [0.44]
	$p = 0.003^\dagger$	$p = 0.25$	$p = 0.93$
Parity			
0 ($n = 256$)	26.8 [12.3]	1.35 [1.08]	0.63 [0.57]
1–2 ($n = 220$)	28.0 [11.9]	1.26 [1.12]	0.58 [0.47]
≥ 3 ($n = 12$)	21.5 [20.8]	1.78 [2.01]	0.82 [0.62]
	$p = 0.11$	$p = 0.07$	$p = 0.32$
Tobacco use			
Yes ($n = 87$)	30.1 [11.6]	1.22 [0.95]	0.58 [0.46]
No ($n = 401$)	26.7 [12.1]	1.33 [1.19]	0.61 [0.54]
	$p = 0.001^*$	$p = 0.06$	$p = 0.19$
Residency			
Coast (< 500 m) ($n = 466$)	27.2 [12.2]	1.32 [1.11]	0.61 [0.51]
Highland (≥ 500 m) ($n = 22$)	28.3 [7.1]	1.04 [1.01]	0.54 [0.46]
	$p = 0.56$	$p = 0.21$	$p = 0.59$
Gestational age at entry			
< 12 weeks ($n = 172$)	23.0 [12.8]	1.19 [0.80]	0.58 [0.45]
≥ 12 weeks ($n = 316$)	28.5 [10.9]	1.35 [1.18]	0.78 [0.54]
	$p = 0.001^*$	$p = 0.02^*$	$p = 0.02^*$
Season at entry			
Spring ($n = 48$)	33.3 [21.8]	1.55 [1.27]	0.65 [0.55]
Summer ($n = 290$)	29.1 [9.4]	1.36 [1.02]	0.60 [0.48]
Autumn ($n = 117$)	20.7 [8.5]	1.26 [0.99]	0.58 [0.55]
Winter ($n = 33$)	17.2 [9.6]	1.25 [1.46]	0.66 [0.50]
	$p = 0.0001^\dagger$	$p = 0.72$	$p = 0.53$

Provided p values are for intragroup comparisons; significant ones in italics as calculated with the Mann–Whitney* or Kruskal–Wallis[†] test.

IQR: interquartile range.

Table II. β -hCG and PAPP-A levels and correlations with 25(OH)D status.

Parameters	β -hCG levels (ng/ml), median [IQR]	PAPP-A levels (mIU/ml), median [IQR]
Deficient Low 25 (OH) D $n = 113$, 23.2%	1.33 [0.98]	0.65 [0.46]
Insufficient 25 (OH) D $n = 198$, 40.6%	1.32 [1.11]	0.61 [0.53]
Sufficient 25 (OH) D $n = 177$, 36.3%	1.30 [1.27]	0.59 [0.55]
	$p = 0.73^*$	$p = 0.64^*$
Rho Spearman coefficient correlations with 25(OH)D levels	0.05 ($p = 0.27$)	–0.06 ($p = 0.21$)

Deficient = (< 20 ng/ml); Insufficient: (20–20.99 ng/ml); Sufficient (≥ 30 ng/ml).

* p value for the intragroup comparison as determined with the Kruskal–Wallis test.

receptor antagonist blocked 1,25(OH)₂D-mediated up-regulation of the 24-hydroxylase gene but did not affect 1,25(OH)₂D stimulated hCG secretion [7].

In conclusion, first trimester 25(OH)D, β -hCG, and PAPP-A levels increase with gestational age; however placental peptides levels do not correlate with vitamin D

levels, suggesting a non-placental 25(OH)D production. Further studies are needed to delineate metabolites regulating serum β -hCG and PAPP-A levels and precise the role of the active hormone 1,25(OH)₂D in this regard.

Declaration of interest: The study was partially supported by the B/024535/09 AECID ('Agencia Española de Cooperación Internacional para el Desarrollo') grant from the Spanish 'Ministerio de Asuntos Exteriores y Cooperación' and the 'Fundación Progreso y Salud' PI-0359 grant from the 'Junta de Andalucía'.

References

- Pérez-López FR. Vitamin D: the secosteroid hormone and human reproduction. *Gynecol Endocrinol* 2007;23:13–24.
- Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266–281.
- Lucas RM, Ponsonby AL, Pasco JA, Morley R. Future health implications of prenatal and early-life vitamin D status. *Nutr Rev* 2008;66:710–720.
- Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placental-decidual function. *J Soc Gynecol Investig* 2004;11:263–271.
- Evans KN, Nguyen L, Chan J, Innes BA, Bulmer JN, Kilby MD, Hewison M. Effects of 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ on cytokine production by human decidual cells. *Biol Reprod* 2006;75:816–822.
- Liu N, Kaplan AT, Low J, Nguyen L, Liu GY, Equils O, Hewison M. Vitamin D Induces innate antibacterial responses in human trophoblasts via an intracrine pathway. *Biol Reprod* 2009;80:398–406.
- Avila E, Díaz L, Barrera D, Halhali A, Méndez I, González L, Zuegel U, Steinmeyer A, Larrea F. Regulation of vitamin D hydroxylases gene expression by 1,25-dihydroxyvitamin D₃ and cyclic AMP in cultured human syncytiotrophoblasts. *J Steroid Biochem Mol Biol* 2007;103:90–96.
- Barrera D, Avila E, Hernández G, Méndez I, González L, Halhali A, Larrea F, Morales A, Díaz L. Calcitriol affects hCG gene transcription in cultured human syncytiotrophoblasts. *Reprod Biol Endocrinol* 2008;6:3.
- Díaz L, Noyola-Martínez N, Barrera D, Hernández G, Avila E, Halhali A, Larrea F. Calcitriol inhibits TNF- α -induced inflammatory cytokines in human trophoblasts. *J Reprod Immunol* 2009;81:17–24.
- Agencia Estatal de Meteorología. Valores climatológicos de Almería. <http://www.aemet.es/es/elclima/datosclimatologicos/valoresclimatologicos?l=63250&k=and> Last accessed 20 October 2010.
- Leino A, Turpeinen U, Koskinen P. Automated measurement of 25-OH vitamin D₃ on the Roche Modular E170 Analyzer. *Clin Chem* 2008;54:2059–2062.
- Siemens Healthcare Diagnostics Products Ltd. www.medical.siemens.com/. Last accessed 20 October 2010.
- Pérez-López FR, Cañas E, L'Hermite M, López E, Roncero MC, Robyn C. Prolactin and human chorionic gonadotrophin (HCG) in amniotic fluid during the two last trimesters of pregnancy. *Int Res Commun* 1974;2:1101–1102.
- Pujol-Amat P, Gamissans O, Cabero L, Pérez-López FR, Benito E, Calaf J, Robyn C. Effects of synthetic luteinizing hormone-releasing hormone on serum gonadotrophins, prolactin and chorionic somatomammotrophin during the last trimester of pregnancy. In: Salvadori B, editor. *Therapy of fetoplacental insufficiency*. Berlin: Springer Verlag; 1975. pp 246–249.
- Pérez-López FR, Tierz JA, Abós MD, Pellejero S, Teijeiro J. Dopaminergic regulation of human prolactin, ACTH, aldosterone, TSH, placental lactogen, chorionic gonadotropin and estriol during pregnancy. In: Endroczi E, Angelucci L, Scapagnini U, de Wied U, editors. *Neuropeptides, neurotransmitters and regulation of endocrine processes*. Budapest: Akadémiai Kiadó; 1982. pp 459–466.
- Bersinger NA, Keller PJ, Naiem A, Fischer M, Schneider H. Pregnancy-specific and pregnancy-associated proteins in threatened abortion. *Gynecol Endocrinol* 1987;1:379–384.
- Casart YC, Tarrazzi K, Camejo MI. Serum levels of interleukin-6, interleukin-1beta and human chorionic gonadotropin in pre-eclamptic and normal pregnancy. *Gynecol Endocrinol* 2007;23:300–303.
- Calleja-Agius J, Schembri-Wismayer P, Calleja N, Brincat M, Spiteri D. Obstetric outcome and cytokine levels in threatened miscarriage. *Gynecol Endocrinol* 2011;27:121–127.
- Dugoff L, Hobbins JC, Malone FD, Porter TF, Luthy D, Comstock CH, Hankins G, Berkowitz RL, Merkatz I, Craigo SD, et al. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). *Am J Obstet Gynecol* 2004;191:1446–1451.
- Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 2008;31:618–624.
- Bayes-Genis A, Conover CA, Overgaard MT, Bailey KR, Christiansen M, Holmes DR Jr, Virmani R, Oxvig C, Schwartz RS. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. *N Engl J Med* 2001;345:1022–1029.
- Krantz DA, Larsen JW, Buchanan PD, Macri JN. First-trimester Down syndrome screening: free β -human chorionic gonadotropin and pregnancy-associated plasma protein A. *Am J Obstet Gynecol* 1996;174:612–616.
- Ramlau-Hansen CH, Moeller UK, Bonde JP, Olsen J, Thulstrup AM. Are serum levels of vitamin D associated with semen quality? Results from a cross-sectional study in young healthy men. *Fertil Steril* 2011;95:1000–1004.
- Shin JS, Choi MY, Longtine MS, Nelson DM. Vitamin D effects on pregnancy and the placenta. *Placenta* 2010;31:1027–1034.
- Equils O, Hewison M. A role for vitamin D in placental immunology. *J Infect Dis* 2010;201:1950–1951.
- Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E, Haddad JG. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. *J Clin Endocrinol Metab* 1986;63:954–959.
- Zerwekh JE. The measurement of vitamin D: analytical aspects. *Ann Clin Biochem* 2004;41:272–281.
- Lewis S, Lucas RM, Halliday J, Ponsonby AL. Vitamin D deficiency and pregnancy: from preconception to birth. *Mol Nutr Food Res* 2010;54:1092–1102.
- Pérez-López FR, Fernández-Alonso AM, Ferrando-Marco P, Salmerón-González MD, Dionis-Sánchez EC, Fiol-Ruiz G, Chedraui P. First trimester serum 25-hydroxyvitamin D status and factors related to lower levels in gravids living in the Spanish Mediterranean coast. *Repr Sci*, in press.
- Sennels HP, Jørgensen FS, Sørensen S. Biological variation of free β chorionic gonadotropin and pregnancy-associated plasma protein A in first trimester pregnancies. *Clin Chem Lab Med* 2010;49:291–295.
- Bischof P, DuBerg S, Herrmann W, Sizonenko PC. Pregnancy-associated plasma protein-A (PAPP-A) and hCG in early pregnancy. *Br J Obstet Gynaecol* 1981;88:973–975.
- Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. *Endocrinol Metab Clin North Am* 2010;39:243–253.