

Assessment of the relationship of vitamin D with serum antioxidant vitamins E and A and their deficiencies in Iranian pregnant women

Zatollah Asemi, MSc, Mohsen Taghizadeh, MSc, Shadi Sarahroodi, Pharm.D, PhD, Shima Jazayeri, MD, PhD, Zohreh Tabasi, MD, Fariba Seyyedi, BS.

ABSTRACT

الأهداف: تحديد العلاقة بين فيتامين د والفيتامينات المضادة للأكسدة هو أ وكذلك العلاقة بين نقص هذه الفيتامينات في النساء الحوامل.

الطريقة: أُجريت هذه الدراسة المقطعية في عيادة النساء والولادة بعيادات ناغافي الشاملة التابعة لجامعة كاشان للعلوم الصحية، كاشان، إيران خلال الفترة من إبريل 2008م إلى أغسطس 2009م. شارك في الدراسة 147 امرأة حامل حيث تم قياس مستويات الفيتامينات د، أ، هـ في مصل الدم وذلك خلال الشهور 5-9 من الحمل. لقد تم تحديد مدى انتشار نقص هذه الفيتامينات بواسطة القيمة القاطعة (cutoff value)، ومن ثم تم الكشف عن العلاقة فيما بينها. تمت مقارنة البيانات بواسطة تحليل التفاوت ومعامل ارتباط بيرسون الخطي، كما كشفت هذه المقارنة عن وجود اختلاف واضح بين المجموعات والذي تم اعتباره مرتفعاً من الناحية الإحصائية ($p < 0.05$).

النتائج: أظهرت الدراسة بأن مستويات تركيز فيتامين د في مصل الدم كانت: 7.2 ± 2.31 ، 17.72 ± 5 ، 33.05 ± 0.94 ميكروغرام/مليتر، وكانت مستويات تركيز فيتامين هـ في مصل الدم كالتالي: 2.15 ± 1.3 ، 8.07 ± 2.3 ميكروغرام/مليتر، فيما كانت مستويات فيتامين أ: 0.16 ± 0.05 ، 0.046 ± 0.013 . وقد قمنا باستخدام القيمة القاطعة من أجل تحديد نسب نقص هذه الفيتامينات في مصل الدم. لقد وصلت نسبة نقص فيتامين د في مصل الدم إلى 95.8%، وكانت نسبة نقص فيتامين أ 7%، ونسبة نقص فيتامين هـ 58.6%. وأسفرت نتائج معامل الارتباط بين فيتامين أ و هـ عن وجود علاقة عكسية فيما بينهما ($r = 0.24$ ، $p = 0.004$).

خاتمة: يعد النقص الحاد للفيتامينات أ، د، هـ بين النساء الحوامل من المشاكل الصحية الخطيرة في مدينة كاشان بإيران.

Objectives: To assess the relationship between vitamin D and serum antioxidant vitamins E and A, and their deficiencies in pregnant women.

Methods: This cross-sectional study was carried out in the maternity clinic of Naghavi Specialists and

Subspecialties Polyclinic, affiliated to Kashan University of Medical Sciences Kashan, Iran between April 2008 and August 2009. One hundred and forty-seven pregnant women were recruited. Vitamin D, A, and E status were assessed using serum at 5-9 months of pregnancy. The prevalence of vitamin deficiency was reported using defined cutoff values. Correlations between vitamin D and vitamins A and E were reported. Data were compared using the analysis of variance and coefficient correlation linear Pearson's. The relative difference between the groups were considered statistically significant ($p < 0.05$).

Results: Serum concentrations of vitamin D were 7.2 ± 2.31 , 17.72 ± 5 and 33.05 ± 0.94 $\mu\text{g/ml}$, for vitamin E 2.15 ± 1.3 and 8.07 ± 2.3 $\mu\text{g/ml}$, and for vitamin A 0.16 ± 0.05 and 0.46 ± 0.13 . We used the defined cutoff values in determining vitamins D, A, and E deficiency. The prevalence of vitamin D deficiency was 95.8%, vitamin A was 7%, and vitamin E was 58.6%. The correlation coefficient between serum vitamin D and vitamin E shows an inverse relationship ($r = 0.24$, $p = 0.004$).

Conclusions: Extent vitamins D, A, and E deficiency among pregnant women is a major public health problem in Kashan, Iran.

Saudi Med J 2010; Vol. 31 (10): 1119-1123

From the Food and Drug Administration (Asemi, Taghizadeh), Kashan University of Medical Sciences, Department of Gynecology and Obstetrics (Tabasi), School of Medicine, Department of Gynecology and Obstetrics (Seyyedi), Kashan University of Medical Sciences, Kashan, Department of Physiology and Pharmacology, School of Medicine (Sarahroodi), Qom University of Medical Sciences, Qom, School of Hygiene and Nutrition (Jazayeri), Iran University of Medical Sciences, Tehran, Iran.

Received 26th June 2010. Accepted 6th September 2010.

Address correspondence and reprint request to: Dr. Shadi Sarahroodi, Department of Physiology and Pharmacology, School of Medicine, Qom University of Medical Sciences, Qom, Iran. Tel. +98 (251) 7737923. Fax. +98 (251) 7831371. E-mail: sarahroodi@yahoo.com

Vitamins are organic compounds and the cause cannot be synthesized by humans, it should be ingested to prevent metabolic disease. Inadequate intake of several vitamins is the main reason of some chronic disease.¹ Ideally, micronutrient deficiencies should be treated before pregnancy. This will help to improve fertility and maternal health.² Micronutrient deficiency is recognized as a major public health problem in women of reproductive age in many developing countries,³⁻⁵ and 50% of pregnancies are unplanned that causes late entry into healthcare.² Also, pregnancy may increase vitamin requirements.¹ Vitamin D is an essential agent in the body. It is a fat soluble vitamin and modulator of calcium metabolism in children and adults.^{6,7} Vitamin D deficiency in pregnancy can have adverse consequences on fetal and maternal health, because there is a significant relationships between maternal and neonatal vitamin D levels.⁸ Vitamin D deficiency may increase risks of cancer (colon, breast and prostate), chronic inflammatory disease, autoimmune diseases (including multiple sclerosis, type I diabetes, inflammatory bowel disease), and metabolic disorders (metabolic syndrome, hypertension) although it increase risks of skeletal disorders such as osteoporosis.⁹⁻¹¹ Low levels of vitamin D may be a risk factor for preeclampsia, low birth weight, neonatal hypocalcaemia, poor postnatal growth, bone fragility, and increased incidence of autoimmune diseases.^{6,12-14} Vitamin E is a fat soluble vitamin and known as a potent antioxidant¹⁵ composed of 8 related compounds (tocopherols and tocotrienols).¹ It is needed in human immune function, and supplementation with this vitamin enhances cell-mediated immunity in old patients.¹⁶⁻¹⁸ Vitamin E deficiency is rare. Muscle weakness, ataxia, and hemolysis are the clinical manifestations of vitamin E deficiency.¹⁹ Vitamin E supplementation may help reduce the risk of some complications in pregnant women involving oxidative stress, such as preeclampsia.²⁰ Also there are some evidences that Vitamins E and C supplementation reduce the endothelial activation and placental dysfunction.²¹ Vitamin A is a fat soluble agent that refers to retinoid compounds.¹ This vitamin is an essential agent in vision (particularly night vision), the immune response, and epithelial cell growth and repair. Its deficiency is marked by xerophthalmia, night blindness, and increased disease susceptibility.^{22,23} Vitamin A deficiency in pregnancy is cause of adverse maternal, fetal and neonatal outcomes and may be the reason of anemia.² The most known functional effect of vitamin A deficiency during pregnancy is transitory blindness, especially during the third trimester of pregnancy.²⁴ It is believed that high serum status of vitamins A and E interact with vitamin D. Most researchers agree on existence of nutritional relationships among fat-soluble vitamins in general,

and particularly nutritional interactions among three vitamins A, D, and E. But there are considerable disagreement as to whether it is due essentially to an interaction among the three vitamins in the intestinal tract, prior to or during absorption, or after absorption in the tissues of the animals.^{25,26} The aim of this study is to determine the serum status of vitamin A, D and E and relationship among them in some pregnant women with different serum status of vitamin D in Kashan city, Iran.

Methods. This cross-sectional study was carried out in the maternity clinic of Naghavi Specialists and Subspecialities Polyclinic, affiliated to Kashan University of Medical Sciences, Kashan, Iran between April 2008 and August 2009. One hundred and forty-seven pregnant women were recruited and completed the interviews (aged 18-35 years). Vitamin D, A, and E status were assessed using serum at 5-9 months of pregnancy.

Pregnant women were excluded if one of the following were diagnosed: multiple pregnancies, maternal hypertension, liver or renal disease, gestational diabetes, drug intake (other than vitamins), and using sunscreen creams. The inclusion criteria during this study were as follows: 5-9 months of pregnancy and age between 18-35 years. Kashan University of Medical Sciences Ethics Committee approved the study and written consent was obtained from all participants.

Data was collected by questionnaire. The questionnaire was translated to Persian by a trained interviewer on or before enrollment. The questionnaire includes socio-demographic questions, reproductive and medical histories, anthropomorphic, and behavioral characteristics. We collected the blood samples (5 ml) in the Reference Laboratory of Kashan, Iran. After separation of serum, the serum samples were sent to Nour Laboratory, Tehran, Iran. Serum concentration of 25-hydroxy vitamin D was used to determine the vitamin D status by immunoassay method. Serum vitamin A and vitamin E were determined simultaneously by a reverse-phase HPLC (high-performance liquid chromatography) attached to the Auto Sampler (717 Plus AS, Waters) (procedure described by Yamini et al²⁷). The internal standards used were all-trans-ethyl- β -apo-8'-carotenoate. The HPLC column (Allsphere ODS-2, 3 μ m, 150 x 4.6 mm, Alltech Associate) was washed by the mobile phase (84% acetonitrile, 14% tetrahydrofuran, 6% methanol (added 0.2% ammonium acetate), and 0.1% triethylamine, and the quality control maintained repeating analyses of pooled reference standards and standard reference material. The assessment of method's precision and accuracy was through Micronutrients Measurement Quality

Assurance Program of Round Robin Proficiency tested by National Institute of Standards and Technology, in which 12 “unknown” samples were analyzed and submitted yearly. For classification purposes, previously published cutoffs were selected. For vitamin D, serum concentrations 32-100 ng/ml (80-250 nmol/L) was safe range during pregnancy, 11-32 ng/ml (27.5-80 nmol/L) was defined as insufficiency, and <10 ng/L (27.5 nmol/L) was considered as indication of hypovitaminosis D or vitamin D deficiency.⁶ For vitamin A: <0.2 µg/ml was considered vitamin A deficiency (normal ≥0.2 µg/ml).²⁸ and for vitamin E: <5 µg/ml was considered as deficiency (normal: ≥5 µg/ml).²⁹

Statistical analysis. Comparisons between groups were made using the one-way analysis of variance. Following a significant F value, post-hoc analysis (Scheffe) was performed to assess specific group comparisons. A difference with $p < 0.05$ between the groups was considered statistically significant. Calculations were performed using the Statistical Package for Social Sciences Version 16 (SPSS Inc., Chicago, Illinois, USA).

Results. One hundred and forty-seven pregnant women at 5-9 months gestation participated in the study. Table 1 summarizes the mean and the percentile of serum concentrations of vitamin D among Iranian pregnant women in the last 4 months of gestation using the defined cutoff values.⁶ The mean and percentile of serum concentrations of vitamin E among Iranian pregnant women in the last 4 months of gestation are summarize in Table 2. Deficiency of vitamin A (vitamin A <0.2 µg/ml) visited only in 10 (7%) of pregnant women (Table 3). Figure 1 shows the correlation between serum vitamin D and vitamin E ($r = 0.24$) and the reverse relationship between serum vitamin A and E in pregnant women at 5-9 months gestation.

Discussion. In the present study, we documented that the prevalence of vitamins D, E, and A deficiencies was common among women in the 4 last months of pregnancy in Iran (Kashan city), likely reflecting lower exposure sunlight for vitamin D deficiency³⁰⁻³¹ and dietary inadequacy in their pregnancy period.²⁸ The assessment of micronutrient status (vitamins and minerals) is complicated during pregnancy, cause there is a general lack of pregnancy-specific laboratory indices for nutritional evaluation,³² although that pregnancy may alter these values, too.³³ Also, vitamin deficiency prevalence may vary between studies; because there is no standardization of the assay and different cutoff values to define deficient status.²⁸ In this study, approximately 95% of these pregnant women had evidence of vitamin D insufficiency and deficiency (serum vitamin D

Table 1 - Mean and percentile of serum concentrations of vitamin D among Iranian pregnant women in the last 4 months of gestation.

Vitamin D status	n	(%)	Mean±SD
Vitamin D deficiency (<10ng/ml)	50	(35.21)	7.2 ± 2.31
Vitamin D insufficiency (11-32 ng/ml)	86	(60.57)	17.72 ± 5
Normal vitamin D (32-100 ng/ml)	6	(4.22)	33.05 ± 0.94
Total	142	(100)	14.66 ± 7.55

Insufficiency is defined as vitamin D 11-32 ng/ml and deficiency as <10 ng/ml. Two samples were missing due to error 2.

Table 2 - Mean and percentile of serum concentrations of vitamin E among Iranian pregnant women in the last 4 months of gestation.

Vitamin E status	n	(%)	Mean±SD
Vitamin E deficiency (<5 µg/ml)	85	58.63)	2.15 ± 1.3
Normal vitamin E (≥5 µg/ml)	60	(41.37)	8.07 ± 2.3
Total	145	(100)	4.6 ± 3.45

Deficiency is defined as vitamin E <5 µg/ml. Five samples were missing due to error 2.

Table 3 - Mean and percentile of serum concentrations of vitamin A among Iranian pregnant women in the last 4 months of gestation.

Vitamin A status	n	(%)	Mean±SD
Vitamin A Deficiency (<0.2 µg/ml)	10	(6.99)	0.16 ± 0.05
Normal Vitamin A (≥0.2 µg/ml)	133	(93.01)	0.46 ± 0.13
Total	143	(100)	0.44 ± 0.15

Deficiency is defined as vitamin A <0.2 µg/ml. Four samples were missing due to error 2.

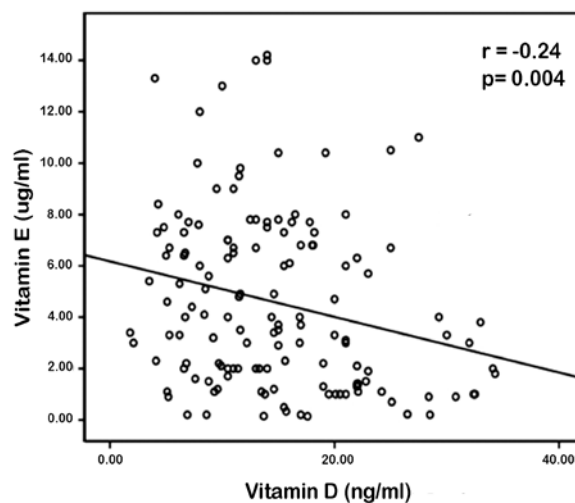


Figure 1 - Relationship of serum vitamin E to serum vitamin D in pregnant women ($r = -0.24$).

concentrations <32 ng/mL [80 nmol/L]); the average of vitamin D concentrations in this group was 13.85±6.63. These findings were higher compared with other studies.^{30,34-36} Vitamin D deficiency during pregnancy is a global problem; researchers have reported a vitamin D deficiency prevalence that ranges from 18-84% in different countries worldwide and by different clothing customs.^{30,34-36} Another study in Iran showed 80% of vitamin D deficiency among women at term delivery time in 2001.³⁰ Two recent studies showed that 30-% of pregnant women in UK³⁷ and 46% in African America⁶ had evidence of vitamin D deficiency. Similar results were obtained in the study of Irene et al,³⁸ who reported 84% Turkish women suffering of vitamin D deficiency.³⁸ Researchers believe that the highest rates of vitamin D deficiency (approximately 80%) occur among dark-skinned or veiled pregnant women.³⁷ There are some evidences that the cause of skin production of vitamin D reduces by reduction of sunlight exposure, its serum status declines in winter mostly at latitudes of >42°N,⁶ although that in some studies season of blood sampling doesn't show a significant trend in vitamin D concentration.³⁸ In this study group, vitamin D deficiency will decrease 90.1% if we change the cutoff to 20 ng/ml for pregnant women. By this cutoff, vitamin D deficiency among African-American pregnant women that were 46% will become to 90%.⁶ Also, we believe that there is an urgent need to a uniform value for these micronutrients in the world. Vitamin D deficiency was prevalent among veiled women and those who loves to stay-at-home because of the hot weather. Kashan city is clear and sunny. Ousr study revealed that only 7% pregnant women had a vitamin A deficiency. The data from Nepali pregnant women was same as our study and showed 6.8% of vitamin A deficiency,²⁸ but it was higher in rural pregnant women from Bangladesh (18.5%)³⁹ and western Rajasthan in India (8.8%).⁴⁰ We believe that the cause of vitamin A deficiency is due to the low intake of this vitamin. Therefore, we recommend a vitamin A supplementation in women with this deficiency. In other part of our study, we discussed that the percentage of vitamin E deficiency in pregnant women was 58.6%. The results from Jiang et al²⁸ indicate that vitamin E deficiency in pregnant women was 25.3%. Vitamin E deficiency is rare primarily and could be seen in special situations resulting in fat malabsorption, including cystic fibrosis, chronic cholestatic liver disease, abetalipoproteinemia, and short bowel syndrome. Muscle weakness, ataxia, and hemolysis are the clinical manifestations of this vitamin deficiency.¹⁹

Vitamin E has an extensive role in oxidative stress⁴¹⁻⁴⁴ and the reduction of protective nutritional^{45,46} and some

of researchers⁴⁷ but not all,⁴⁸ enzymatic antioxidant defenses. Therefore, it is estimated that this deficiency is due to high oxidative stress and its low intake of foods. Thus, we recommend vitamin E supplementation in pregnant women with this kind of deficiency. The last part of our study showed an antagonism between vitamin D and E serum status in pregnant women. Rohde and DeLuca²⁶ revealed a reverse relationship between serum vitamin D and A in rats. There were no any difference found between serum status of vitamin A and D. The main source of vitamin D and E are fatty tissue and both of them are carried by same carrier in the blood,⁴⁹ therefore, this can be the reason of reverse relationship between these 2 vitamins. We did not find any relation between vitamin A and vitamin E or vitamin D. Although that there are some evidences that shows vitamin A antagonizes vitamin D by affecting the activity of the calciferol-metabolizing enzymes.²⁵ But the different of our result could be because of the main source of vitamin A, which is liver and for vitamin D and E, it is fatty tissue and their carrier is not the same too.⁴⁹

Limitation of this study, we cannot control the participants on their diet and stay under the sunlight, and we were unable to get another blood sample from any of the participant.

Acknowledgment. The authors would like to thank the staff of Naghavi Clinic, Kashan, Iran for their assistance in this project.

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