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Original

Association between serum and follicular fluid vitamin D levels and pregnancy rate in women undergoing in vitro fertilization/intracytoplasmic sperm injection

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Abstract

Introduction: There are controversies about relationship between serum and follicle vitamin D levels and in vitro fertilization (IVF) outcomes.

Objectives: The goal of this study was to determine association between serum and follicular vitamin D levels and IVF outcome in the Iranian population.

Patients and Methods: This cohort study was conducted in a women's hospital. One hundred and sixty patients (mean age = 28 years) who were candidates (by simple sampling) for IVF enrolled in the study. Levels of 25 (OH)-D in follicular fluid and serum were measured. Biochemical pregnancy was determined by detecting serum β-hCG. A detectable gestational sac was considered as a characteristic of clinical pregnancy.

Results: Biochemical pregnancy was detected in 49 (30.6) and clinical pregnancy in 39 (24.4). Mean vitamin D levels of serum and follicular fluid were not significantly different between cases that had clinical pregnancy or not. In cases with serum vitamin D level less than 30, mean follicular vitamin D level and body mass index (BMI) were significantly lower than the cases with serum vitamin D level more than 30. Mean serum vitamin D levels, biochemical and clinical pregnancy rates were significantly different in cases with follicular vitamin D level less or equal/more than 30. Logistic regression by considering biochemical pregnancy as dependent variable and other variables as independent variables showed that the only independent predictor was grade of embryo

Conclusion: Follicular vitamin D level could affect pregnancy outcome in IVF cycles while serum vitamin D levels do not play any role in the IVF outcome.

Keywords: IVF, Vitamin D, Pregnancy

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Introduction

Infertility is defined as inability to conceive after 1 year of unprotected sex that affects approximately 20 of couples (1). Along with increase of infertile couples, application of assisted reproductive technology (dois) increases.

Vitamin D is a steroid hormone that is involved in calcium-phosphate homeostasis and bone metabolism (2,3). On the other hand, autoimmunity, insulin resistance, cardiovascular disease, and malignancies are among other diseases in which vitamin D has a crucial role (3,4). Along with physiologic processes that are mediated with vitamin D, its role in reproductive physiology is under investigation. Vitamin D deficiency is prevalent in women of reproductive age (5,6).

Previous studies are evidence of vitamin D effects upon placental steroidogenesis, calcium transport through the placenta, expression of placental lactogen, and decidualization of the endometrium (3,7). On the other hand, vitamin D regulates genes that control implantation and establishment of the fetoplacental component (7-9).

There are controversies about relationship between serum and follicular fluid vitamin D levels and in vitro fertilization (IVF) outcomes (10-12).

Objectives

The goal of this study was to determine association between serum and follicular fluid vitamin D levels and IVF outcome in the Iranian population.

Patients and Methods

Study design

This cohort study was conducted in Yas hospital, (affiliated to Tehran University of Medical Sciences) between March 2015 and March 2016. Inclusion criteria were: maternal

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Implication for health policy/practice/research/medical education

There are controversies about relationship between serum and follicular fluid vitamin D levels and IVF outcomes. In a study on 90 cases, who were candidates for IVF, we found follicular vitamin D level can affect pregnancy outcome in IVF cycles while serum vitamin D levels do not play any role in the IVF outcome. It is necessary to use vitamin D supplementation to control and treat vitamin D deficiency.

age between 18 and 35, body mass index (BMI) between 18 and 25. Exclusion criteria were: history of cancer, MS, hypertension, diabetes, autoimmune disease, liver, coronary and kidney diseases. If retrieved oocytes were more than 15 or less than 2, estradiol level was less than 500 on the day of hCG injection and if cases developed OHSS (ovarian hyper-stimulation syndrome) during cycle they were excluded from the study. If fresh embryos transfer was not possible or there were more than 90 days between transfer and vitamin D evaluation, patients excluded from the study. All patients underwent IVF cycles using standardized regimens (GnRH antagonist). Controlled ovarian hyperstimulation was initiated with recombinant follicle stimulating hormone (r-FSH).

All patients were followed up by vaginal sonography. The ovulation target was two or more follicles greater than 17 mm. Thirty-six hours after human chorionic gonadotropin (hCG) administration (10 000 units), oocytes were picked up. The day after hCG administration, blood samples were collected and stored at -20°C and follicular fluid was collected by follicular puncture and aspiration. Follicular fluid was centrifuged at 3000 g for 15 minutes and the supernatant was stored at -70°C. All retrieved oocytes were fertilized. Embryo transfer was performed under ultrasound guidance on day 3 after insemination. Minimum one and maximum two high-grade blastocysts were transferred transvaginally. Biochemical pregnancy was determined by detecting serum β-hCG. A detectable gestational sac + embryonic pole with or without cardiac activity was considered as a characteristic of clinical pregnancy. The sonography was done at 5th gestational week. Data regarding maternal age, BMI, AMH (anti -Müllerian hormone), progesterone level, endometrial thickness, number of retrieved oocytes, number of transferred embryos and grade of embryos were recorded for all cases.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Tehran University of Medical Sciences approved this study. The institutional ethical committee at Tehran University of Medical Sciences approved all study protocols (IR.TUMS.9211290006). All patients were asked to fill in an informed consent form before any intervention. This study was extracted from M.D thesis Samaneh Saghafian Larijani at this university.

Data analysis

We considered vitamin D level less than 30 as cut off point of insufficient and deficient values. Vitamin D level was determined by ELIZA method. SPSS version 22 was used for data analysis. Student’s t test was used for comparison of continuous variables. Correlation coefficient was calculated for continuous variables. Logistic regression by considering biochemical and clinical pregnancies as dependent and other variables as independent was performed. *P* value of less than 0.05 was considered statistically significant.

Results

One hundred and sixty patients were enrolled in the study. Demographic and laboratory characteristics are summarized in Table 1. Biochemical pregnancy was detected in 49 (30.6) and clinical pregnancy in 39 (24.4). Mean vitamin D levels of serum and follicular fluid were not significantly different between cases that had clinical

Table 1. Demographic and laboratory characteristics

Variables	Mean ± SD/ No. (%)
Age (year)	28.1±4.2
BMI (kg/m^2)	20.9±3
AMH	
<0.3	12 (7.5)
0.3-0.6	29 (18.1)
0.7-0.9	34 (21.3)
1-3	48 (30)
>3	37 (23.1)
FSH	
<10	130 (81.3)
10-12	24 (15)
12-15	6 (3.8)
LH	
<10	141 (77.8)
10-12	16 (10)
12-15	3 (1.9)
Estradiol	
<400	5 (3.1)
400-1000	32 (20)
1000-2000	69 (43.1)
2000-3000	29 (18.1)
>3000	25 (15.6)
Progesterone (ng/mL)	
<0.5	25 (15.6)
0.5-0.9	94 (58.8)
≥1	41 (25.7)
Endometrial thickness (mm)	
5-7	3 (1.9)
7-8	63 (39.4)
8-9	43 (26.9)
9-10	36 (22.5)
10-12	15 (9.4)
Serum vitamin D level (nmol/L)	40.6±16.4
Follicular fluid vitamin D level (nmol/L)	45.1±18.3

pregnancy or not (Table 2). In cases with serum vitamin D level less than 30, mean follicular vitamin D level and BMI were significantly lower than the cases with serum vitamin D level more than 30 (Table 3). Mean serum vitamin D levels, chemical and clinical pregnancy rates were significantly higher in cases with sufficient follicular vitamin d level (Table 4).

Logistic regression by considering biochemical pregnancy as dependent variable and other variables as independent variables showed that the only independent predictor was grade of embryo (Table 5). Correlation coefficient between serum vitamin D and age was r=-0.03, $P=0.6$, while the correlation coefficient between serum vitamin D and BMI was r=0.06, $P=0.4$. Moreover, the correlation coefficient between follicular vitamin D and age was r=0.04, $P=0.5$, and we found the correlation coefficient between follicular d and BMI was r=0.05, $P=0.5$ (Table 6).

Discussion

The literature in the form of systematic reviews revealed the close link between the serum level of vitamin D and the prognosis of IVF/ICSI; however, despite determining the pointed significance, the results had no enough validity because of a great heterogeneity across the studies. As shown by Zhao et al in 2018 in a meta-analysis (13), the deficiency in vitamin D was linked to reduce the chance for clinical pregnancy and thus live birth after IVF/ICSI and in this regard, supplementing vitamin D to achieve requested prognosis following this procedure is highly recommended. However, their result was ultimately accompanied by a significant heterogeneity. Moreover, to the best of our knowledge, a few studies focused on the association between follicular vitamin D level and pregnancy rate following IVF emphasizing more studies for confirming such association. In this context, we aimed to assess the serum as well as follicular levels of vitamin D and outcome of IVF/ICSI adjusting baseline characteristics. The result of this study showed that mean vitamin D level in women with and without clinical pregnancy was not significantly different and pregnancy rates were not significantly different between women with and without normal serum vitamin D level. More interestingly, the results also indicated that

Table 2. Comparison of factors between cases with and without pregnancy

	With clinical pregnancy	Without clinical pregnancy	*P* value
Age	29.4±3.9	28.7±4.2	0.3
BMI	20.5±2.9	21±3	0.3
Serum vitamin D (nmol/L)	43.6±15.8	39.6±16.5	0.1
Follicular vitamin D (nmol/L)	47.9±16.8	44.2±18.8	0.2
Transferred embryos	2.5±0.7	2.6±0.7	0.3

Table 3. Comparison of values in cases with serum vitamin D level less than or equal/more than cut off point

	Serum vitamin D level <30 n = 46 (28.8%)	Serum vitamin D level ≥30 n = 114(71.3%)	*P* value
Age	29.4±3.9	28.6±4.2	0.1
Retrieved oocytes	9.3±12.2	6.3±3.9	0.1
Follicular vitamin D (nmol/L)	27.8±8.5	52.1±16.5	<0.001
BMI (kg/m^2)	20.2±2.5	21.2±3.1	0.04
Chemical pregnancy	11(23.8%)	38(32.3%)	0.2
Clinical pregnancy	10(21.7%)	29(25.4%)	0.1

Table 4. Comparison of values in cases with follicular vitamin D level less than or equal/more than cut off point

	Follicular vitamin D level <30 n = 40 (24.8%)	Follicular vitamin D ≥30 n = 120 (75.1%)	*P* value
Age (year)	28.5±3.8	29±4.3	0.5
Retrieved oocytes	9.7±13	7.3±3.8	0.07
Serum vitamin D (nmol/L)	24.9±11.3	45.8±14.3	<0.001
BMI (kg/m^2)	20.4±2.6	21.1±3.1	0.2
Chemical pregnancy	7(17.5%)	42(35%)	0.03
Clinical pregnancy	5(12.5%)	34(28.3%)	0.04

Table 5. Logistic regression by considering chemical pregnancy as dependent variable and other variables as independent variables

	OR	95% CI	*P* value
Maternal age (year)	1	0.9-1	0.8
BMI (kg/m^2)	1.05	0.9-1.1	0.3
Grade of embryo	1.07	11.01-1.1	0.02
Progesterone (ng/mL)	1.1	0.6-1.8	0.6
Serum vitamin D level (nmol/L)	1.1	0.3-3.1	0.8
Follicular vitamin D level(nmol/L)	0.3	0.1-1.2	0.1
Endometrial thickness (mm)	1	0.8-1.1	0.9

Table 6. Logistic regression by considering clinical pregnancy as dependent variable and other variables as independent variables

	OR	95% CI	*P* value
Maternal age (year)	1.03	0.9-1.1	0.3
BMI (kg/m^2)	1.06	0.9-1.2	0.3
Grade of embryo	1.1	1-1.1	0.003
Progesterone (ng/mL)	1.2	0.7-2.2	0.4
Serum vitamin D level (nmol/L)	0.9	0.9-1.03	0.5
Follicular vitamin D level(nmol/L)	0.9	0.9-1.03	0.9
Endometrial thickness (mm)	0.9	0.8-1.1	0.9

biochemical and clinical pregnancies were significantly higher in women with normal follicular vitamin D levels. Therefore, although the serum level of biomarker might not be an indicator for predicting IVF/ICSI outcome, the concentration of this biomarker can powerfully predict IVF/ICSI consequences effectively. Such results, therefore, have some advantages and limitations in clinical setting. Although finding a sensitive biomarker for predicting IVF/ICSI is beneficial, measuring this marker in follicles is not always easily performed. However, almost all similar studies agree on the central role of the follicular vitamin D level in assessing the outcome of IVF/ICSI. As shown by Ciepiela and colleagues in 2018 (14), the level of vitamin D in follicular fluid correlated negatively with the oocytes ability to undergo fertilization and subsequent preimplantation embryo development, but such role could not be demonstrated for low serum vitamin D concentration

Farzadi et al also indicated higher level of follicular vitamin D in women with positive IVF outcome (10). They included 80 women who were candidates for ICSI/IVF. Follicular vitamin D was significantly correlated with patient age. Garbedian et al also evaluated serum vitamin level of 173 women who were candidates of IVF and found that 54.9 had insufficient 25 (OH) D levels. According to their final logistic regression modeling, low- serum vitamin D level was the main predictor for clinical pregnancy (11). Aleyasin et al assessed 82 women who were candidates for doi. They evaluated serum and follicular vitamin D levels. Mean serum and follicular vitamin D levels were significantly different in cases with and without biochemical and clinical pregnancies (15). Such as their study in our study follicular vitamin D level was not correlated with age and BMI. In a study conducted by Anifandis et al, decreased chance of pregnancy was reported by an increase of vitamin D level. They suggested that vitamin D decreases chance of pregnancy by affecting insulin function and decreasing follicular fluid glucose (16). Ozkan et al and Rudick et al showed that vitamin D sufficiency was associated with higher pregnancy rate in recipients of egg donation (3,12).

With respect to the association between vitamin D deficiency and the likelihood of pregnancy following IVF/ICSI, some physiological suggestions have been also systematically discussed in recent studies even in meta-analysis form. Such physiological processes have been also examined in animal model experiments. In a systematic review, Lerchbaum and Obermayer-Pietsch revealed the vitamin D receptor and its related metabolizing enzymes can be found in reproductive tissues. In this regard, knockout of vitamin D receptors is significantly associated with gonadal insufficiency as well as histological abnormalities in uterus and ovary. In addition, vitamin D can influence steroidogenesis and thus estradiol and progesterone production (17). In another systematic review by Irani and Mehri (18), it was indicated that in granulosa cells, vitamin D deficiency can alter signaling related to activation of AMH, FSH sensitivity, and progesterone production and release, and thus vitamin D deficiency may play a major role in impairment of ovarian follicular development and luteinization. Increased endometrial receptivity and chance of pregnancy by binding vitamin D to its receptors in the endometrium was suggested by Ozkan et al as the explanation of increased pregnancy rate in women with sufficient vitamin D level (3). On the other hand, vitamin D may increase the expression of HOXA10 gene which has important role in implantation (15). Vitamin D could modulate endometrial immune response by reducing the decidual T-cell activity, synthesis of cytokines, interleukin 1 and 6 and TNFα which have roles in implantation (16,17). Steroidogenesis, folliculogenesis, implantation and fetus development are other functions related to vitamin D (10). The controversies regarding role of vitamin D in affecting pregnancy outcome in doi cycles could be according to different sample size, different causes of infertility, dissimilar protocols in ovulation induction and different methods of assessment of vitamin D.

Conclusion

Follicular vitamin D level could affect pregnancy outcome in IVF cycles while serum vitamin D levels do not play any role in the IVF outcome.

Limitations of the study

This study had some limitations. It was conducted in a tertiary hospital and cases with different doi protocol included. Larger multi-centric studies are recommended.

Authors' contribution

SSL and ME were the principal investigators of the study. SSL, ME and AA were included in preparing the concept and design. ME and AA revised the manuscript and critically evaluated the intellectual contents. Analysis and interpretation of data was performed by HM and MN. Statistical analysis was performed by HM and Mn. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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