Original Article

Do Serum Vitamin D Levels Have Any Effect on Intrauterine Insemination Success?

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Abstract

Background: Recent studies have shown that vitamin D has an essential role in the reproductive system. In this study, we aimed to investigate the effect of vitamin D levels in patients undergoing ovulation induction (OI), and subsequent intrauterine insemination (IUI) procedure.

Materials and Methods: One hundred and four infertile and one hundred and three fertile women were recruited in this cross-sectional study which was conducted in a tertiary level maternity hospital. Infertile patients were divided into pregnant and non-pregnant subgroups after treatment. Individual characteristics and 25-hydroxyvitamin D3 [25(OH)D3] levels were compared between the groups.

Results: The vast majority of our study population consisted of women who had vitamin D deficiency (96.6%). There was no statistically significant difference between infertile and fertile groups in terms of serum 25(OH)D3 levels (P=0.512). Similarly, no significant difference was observed between the pregnant and non-pregnant subgroups of infertile patients regarding 25(OH)D3 levels (P=0.267).

Conclusion: There is no association between female infertility and serum vitamin D levels. Vitamin D does not predict pregnancy in infertile women undergoing OI with IUI. Further research which will provide a comparison between much more women who have deficient and sufficient 25(OH)D3 levels is warranted.

Keywords: Infertility, Intrauterine Insemination, Ovulation Induction, Vitamin D

Introduction

Intrauterine insemination (IUI) is used to transport sperm directly into the uterus. It is a simple, non-invasive, and cost-effective technique used for assisted reproduction. The most common indication for IUI is cervical infertility, and it is also used in male subfertility, anovulation, endometriosis cases in which at least one tube is healthy, as well as unexplained infertility (1, 2). Although there may be a trend towards higher pregnancy rates when the number of IUIs per cycle is increased, a recent meta-analysis has shown that increased IUI numbers do not increase pregnancy (3). Previous investigations reported that IUI had a success rate of 10-20% for clinical pregnancies (4).

Recently, the effect of vitamin D (VD) has been investigated on not only the musculoskeletal system, but also in the reproductive and other systems (5). The biologic actions of VD are mediated through the vitamin D receptor (VDR). VDR was found to be in the ovary (particularly the granulosa cells), uterus, placenta, and testis, suggesting VD may have a significant role in human reproduction (6). Two studies supporting this data indicated that VD deficiency is responsible for reduced fertility and reproductive capacity in female rats (7, 8). Research conducted on human subjects also supports this role as in experimental animal studies (9).

Calcitriol (1, 25 dihydroxyvitamin D3) which is the active form of VD stimulates CYP19 expression (CYP19 encodes the aromatase enzyme) that results in increased estrogen production, when it was bound to VDR (10). Furthermore, it has been reported that decidua secretes calcitriol during blastocyst implantation, and calcitriol has been reported to regulate the immune response in the maternal-fetal interface during pregnancy (11).

There are several studies which presented controversial results on the differences in 25-hydroxyvitamin D3 [25(OH)D3] levels of the patients undergoing different infertility treatment modalities (12, 13). The aim of our study was to investigate the 25(OH)D3 levels in patients who underwent ovulation induction with IUI and then to...
determine the relationship between 25 (OH) D₃ levels and the occurrence of pregnancy.

Materials and Methods

This case-control study was conducted between March 2014 and June 2014 in the infertility outpatient clinics of Zekai Tahir Burak Women's Health Education and Research Hospital. This is a government supported tertiary level maternity hospital located in the capital city of Turkey. The institutional review board approved the study and informed consent was obtained from each patient (approval number: 23.09.2013/9). All of the study protocols were carried out in accordance with the Helsinki Declaration (14).

We defined the infertile patients as those reproductive age couples who were unable to become pregnant in the absence of contraception. For the women below 35 years of age, infertility was diagnosed as a minimum of 1 year of trying to become pregnant, whilst for the women above 35 years of age, the diagnosis was limited to 6 months of unprotected sexual intercourse. After we obtained detailed information about age, duration of infertility, infertility type, previous history of surgery, and any systemic disturbances (such as diabetes mellitus, hypertension, and thyroidal disease), a complete physical and gynaecological examination was performed on all of the women. We confirmed tubal patency in the women using hysterosalpingography (HSG) and if there was bilateral tubal occlusion detected with HSG, we applied laparoscopy and hysteroscopy to define any pathology such as pelvic adhesions or endometriosis. When we suspected an intra-cavitary lesion in the uterus after HSG, or transvaginal ultrasound, we performed hysteroscopy.

We included women with mild male factor infertility, unexplained infertility, and polycystic ovary syndrome (PCOS). We excluded patients who had advanced age (above 40 years of age), any systemic or endocrine diseases, stage 3-4 endometriosis, or intracavitary lesions in uterus (such as endometrial polyp, submucous myoma, and uterine septum), smokers and women who used any kinds of drugs or substances likely to affect levels of VD. We also excluded patients whose partner had a motile sperm count lower than 5 million/mL. The fertile group consisted of patients who applied to the family planning unit of our hospital for contraceptions. These patients had given birth in the previous 12 months, has not breastfed their neonate, and had no history of infertility.

After initial clinical assessment, infertile patients were evaluated for clomiphene citrate (CC) or gonadotropins (Gn) and IUI use. Those patients who had used CC with IUI treatments for three times or were above 35 years of age were directed into the Gn with IUI regimen (n=63), whilst the other infertile patients were directed into the CC and IUI regimen (n=41). When 18-20 mm (dominant follicles) were found through ultrasound, 2 human chorionic gonadotropin (hCG, Pregnyl, MSD, Netherlands) ampoules containing 5,000 units each, were injected intramuscularly, and IUI applied 36 hours after the injection. When there were 3 or more dominant follicles, or endometrial thickness was less than 6 mm, hCG was not administered. Then 2 weeks later, a blood sample was obtained from patients for β-h-CG measurement. Clinical pregnancy was diagnosed 5 weeks after IUI, when the evidence of fetal heart activity or presence of the gestational sac in the uterine cavity was detected.

The concentration of serum 25 (OH) D₃ was used to determine the status of VD in the body for this study since it has been proven to be the best biomarker for VD insufficiency. It also reflects VD levels from both dietary intake and in-skin synthesis (6). The two groups were matched in term of veiling habits, daily exposure to sunlight, and dietary intake of VD-rich foods which was determined by a dietician.

The serum levels of 25 (OH) D₃ levels and baseline hormones including estradiol, follicle stimulating hormone (FSH), luteinizing hormone, prolactin, and thyroid stimulating hormone were measured on the third day of the menstrual cycle when ovulation induction was started. We performed the recruitment of study volunteers in a single season, because the blood levels of VD have seasonal variabilities (15, 16). In addition, patients living in the same geographical region were selected for the study (17).

After overnight fasting, venous blood samples were obtained early in the morning and transferred to the laboratory in a non-transparant box to avoid exposure to light, and then serum was separated by centrifugation at 5,000 rpm (2,236 g) for 10 minutes. The serum 25 (OH) D₃ levels were measured using an enzyme linked immunosorbent assay kit (Immunodiagnostics AG, Leverkusen, Germany), and presented in ng/mL. The intra-assay and inter-assay coefficients of variation for serum 25 (OH) D₃ were 8.9 and 10.6% respectively. Serum 25 (OH) D₃ concentrations <20 ng/mL was considered as VD deficiency. Types of VD deficiency were also classified as mild (10-20 ng/mL), moderate (5-10 ng/mL), and severe (<5 ng/mL). Serum 25 (OH) D₃ concentrations between 20 and 30 ng/mL was accepted as VD insufficiency whereas a threshold value of ≥30 ng/mL was considered sufficient serum VD levels. Basal hormone levels were measured using an Immulite 2000 analyzer (EURO/DPC Ltd., Gwynedd, UK). Body mass index (BMI) was defined as the weight in kilograms divided by the square of the height in meters.

We examined the women who had a positive result for β-hCG using transvaginal ultrasound at at least weeks 6-7 of gestation to detect fetal cardiac activity. The difference between the two subgroups (pregnant and non-pregnant) of infertile patients in terms of 25 (OH) D₃ levels was the primary outcome measured of this study. The secondary outcome was the comparison of serum 25 (OH) D₃ levels between infertile and fertile groups.

Data were recorded and analysed using the Statistical Package for the Social Sciences program for Windows version 17.0 (SPSS Inc, Chicago, IL, USA). The normal distribution of the variables was assessed using the Shapiro-Wilk's test. Continuous variables were presented as
Table 1: Descriptive characteristics and serum 25 (OH) D levels of infertile and fertile patients

| Characteristic | Infertile group n=104 | Fertile group n=103 | P value |
|----------------|------------------------|----------------------|---------|
| Age (Y)*       | 28.1 (4.7)             | 29.4 (5.4)           | 0.088a  |
| BMI (kg/m²)**  | 25.1 (3.6)             | 25.7 (3.8)           | 0.234a  |
| Gravida'       | 0 (0-5)                | 0 (0-5)              | <0.001b |
| Parity'        | 0 (0-5)                | 0 (0-5)              | <0.001b |
| Alive'         | 0 (0-5)                | 0 (0-5)              | <0.001b |
| Abortion'      | 0 (0-5)                | 0 (0-5)              | <0.001b |
| FSH (mIU/mL)** | 7.4 (2.1)              | 6.2 (1.6)            | 0.001c  |
| LH (mIU/mL)**  | 5.3 (2.5)              | 4.9 (1.9)            | 0.154c  |
| Estradiol (pg/ml)' | 44.0 (12-148) | 42.7 (21-99)        | 0.791b  |
| TSH (mIU/mL)** | 1.9 (0.8)              | 2 (0.9)              | 0.859a  |
| Prolactin (ng/mL)** | 12.2 (4.6) | 14.4 (5.4)          | 0.002c  |
| 25 (OH) D₃ (ng/mL)*** | 7.3 (3-25.5) | 6.8 (3.4-37.1) | 0.512c  |

BMI: Body mass index, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid stimulating hormone, CC: Ovulation induction with clomiphene citrate, Gn: Ovulation induction with gonadotropin, *, **, and ***: Median (minimum-maximum), *, Mean (SD), ’: Student’s t test, and **: Mann Whitney U test. P<0.05 is considered as statistically significant.

Table 2: Individual characteristics, ovulation induction type and vitamin D levels in pregnant and non-pregnant patients after IUI

| Characteristic     | Non-pregnant group n=90 | Pregnant group n=14 | P value |
|--------------------|-------------------------|---------------------|---------|
| Age (Y)*           | 28.5 (4.7)              | 25.5 (4.4)          | 0.027a  |
| BMI (kg/m²)**      | 25.3 (3.5)              | 25.3 (3.4)          | 0.784a  |
| Gravida'           | 0 (0-6)                 | 0 (0-2)             | 0.745a  |
| Parity'            | 0 (0-1)                 | 0 (0-1)             | 0.459a  |
| Alive'             | 0 (0-1)                 | 0 (0-1)             | 0.459a  |
| Miscarriage'       | 0 (0-5)                 | 0 (0-2)             | 0.335a  |
| FSH (mIU/mL)**     | 7.1 (3.5-13.6)          | 6.8 (3.4-13.5)      | 0.378a  |
| LH (mIU/mL)**      | 4.9 (2.1-14)            | 5.9 (2.2-10.3)      | 0.247a  |
| Estradiol (pg/mL)' | 44.5 (12-148)           | 42.5 (20-82)        | 0.398a  |
| TSH (mIU/mL)**     | 1.8 (0.4-5.3)           | 2 (1.3-3.5)         | 0.160a  |
| Prolactin (ng/mL)**| 12.3 (3.9-28.4)         | 12.9 (7.6-20.9)     | 0.788a  |
| 25 (OH) D₃ (ng/mL)*** | 7.3 (3-25.5)        | 8.1 (4.7-22.1)      | 0.267a  |
| Ovulation induction type*** | 55 (61.1)    | 58 (57.1)          | 0.777a  |

BMI: Body mass index, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid stimulating hormone, CC: Ovulation induction with clomiphene citrate, Gn: Ovulation induction with gonadotropin, *, **, and ***: Median (minimum-maximum), *, Mean (SD), ’: Student’s t test, and **: Mann Whitney U test. P<0.05 is considered as statistically significant.

Discussion

Our study showed that infertile and fertile patients had similar serum VD levels and that there was no statistically significant difference in serum VD measurements between the pregnant and non-pregnant groups after IUI.
VD has an essential role in both male and female reproductive system (19). It was found that its deficiency is highly prevalent among women undergoing ovarian stimulation (9). Considering the previous data, we designed such a study assuming that VD could be lower in infertile patients, but we found no relationship between them. This result may be due to the fact that VD deficiency is very common in our study population, because 200 of the 207 patients also including women with no fertility problem had VD deficiency at the initial examination. This was a surprise and suggests that fertile patients who had a delivery in the preceding 12 months may have exhausted their VD stores during the most recent pregnancy and they had not been able to replace it yet. VD deficiency is one of the general public health matters in our country, with similar inferences having been suggested in other studies from our country (20, 21).

Ovulation induction with IUI is the most utilized method of infertility treatment in our unit. The success of IUI treatment is multifactorial, and pregnancy rates per cycle have been estimated as 10.2% in a IUI cycle with controlled ovarian stimulation (22).

A study by Ott et al. (23) demonstrated that 25 (OH) D₃ levels may predict ovarian response to ovarian stimulation. This suggestion is consistent with another study showing that VDR exists in human ovaries and is important for sex steroid synthesis (10). However, when we compared pregnant patients with non pregnant patients in terms of serum VD levels, there was no statistically significant difference between them. This may be associated to the differences of individual VDR receptivity and VDR polymorphism (24). Although VDR polymorphism has been reported as not being related to infertility in an endometriosis study, there is a need for further research to clarify this particular issue (25). Another noteworthy result of our study is that patients who became pregnant after IUI treatment were younger than those who did not. It has already been shown that age is one of the most important factors in infertility management (26).

In two rat studies, VD deficiency was shown to significantly increase infertility, decrease probability of viable births and healthy full-grown individuals (8, 27). Although the exact mechanism remains to be elucidated, compromised ovarian folliculogenesis and infertility were found in two studies conducted on VD deficient mice (28, 29). A recent human study supporting these inferences showed that there is a negative correlation between serum levels of 25 (OH) D₃ and FSH (30). However, we found no correlation between them.

VD has been found to be related with the activation of key enzymes in steroidogenesis such as 3-beta-hydroxysteroid dehydrogenase, and it has been shown to induce the production of progesterone that consequently leads to uterine quiescence (5). Thus, VD may play a protective role for ongoing pregnancies through this mechanism.

A recent randomized controlled trial by Asadi et al. (31) showed that endometrial thickness was enhanced by the administration of VD in patients undergoing Gn and IUI treatment. Another study found that higher serum and follicular fluid 25 (OH) D₃ levels were associated with higher pregnancy rates in women undergoing IVF (32). Similarly, a Greek study group found that follicular fluid VD levels significantly correlated with the quality of embryos (18). However, the same authors suggested that excess serum and follicular fluid vitamin levels may have a detrimental effect on IVF outcomes. These findings led us to think that an optimal level of VD is necessary for ovulation, fertilization, and implantation.

The strength of this study is that our data is single-centered and reliable. The data were obtained prospectively from the patients living in the same geographical region during the same season. There is a limitation to our study; VD deficiency was wide-spread in our study population, consistent with the results of previous studies on this issue (19, 20). The similarity of the groups in terms of high prevalence of VD deficiency may have caused 25 (OH) D₃ levels to not be distinguishable between the groups.

Conclusion

No significant difference was observed between pregnant and nonpregnant women who underwent ovulation induction with IUI treatment with regard to serum 25 (OH) D₃ levels. No association was found between infertility and serum 25 (OH) D₃ levels either. Further research which compares women who have deficient and sufficient serum VD levels is warranted.

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Author’s Contributions

N.Y., E.E., A.T.; Participated in study design, data collection and evaluation, drafting and statistical analysis. A.S., A.S.O.-E.; Contributed to data collection, evaluation and interpretation. S.E., H.I.Y.; Contributed extensively in interpretation of the data, critical revision of the article. All authors performed editing and approving the final version of this paper for submission, also participated in the finalization of the manuscript and approved the final draft.

References

1. Cantineau AE, Cohlen BJ, Al-Inany H, Heineman MJ. Intrauterine insemination versus fallopian tube sperm perfusion for non-tubal infertility. Cochrane Database Syst Rev. 2014; 12: CD006942.
2. Koçak M, Demir B, Yalvaç S, Haberal A. Effectiveness of ovulation induction and intrauterine insemination in heterogenous subfertile couples. Gynecol Obstet and Reprod Med. 2001; 7(2): 99-102.
3. Zavos A, Daponte A, Garas A, Verykouki C, Papanikolaou E, Anifandis G, et al. Double versus single homologous intrauterine insemination for male factor infertility: a systematic review and meta-analysis. Asian J Androl. 2013;15(4): 533-538.
4. Duran HE, Morshed M, Kruger T, Oehninger S. Intrauterine insemination: a systematic review on determinants of success. Hum Reprod Update. 2002; 8(4): 373-384.
Yilmaz et al.

5. Irani M, Merhi Z. Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertil Steril. 2014; 102: 460-468.

6. Johnson LE, DeLuca HF. Vitamin D receptor null mutant mice fed high levels of calcium are fertile. J Nutr. 2001; 131: 1787–1791.

7. Johnson LE, DeLuca HF. Reproductive defects are corrected in vitamin D-deficient female rats fed high calcium, phosphorus and lactose diet. J Nutr. 2002; 132(8): 2270–2273

8. Kwiecinski GG, Petrie GI, DeLuca HF. 1, 25-Dihydroxyvitamin D3 restores fertility of vitamin D-deficient female rats. Am J Physiol Endocrinol Metab. 1989; 256(4): 483–487

9. Vanni VS, Viganò P, Somigliana E, Papaleo E, Paffen O, Pagliari L, et al. Vitamin D and assisted reproduction technologies: current concepts. Reprod Biol Endocrinol. 2014; 12: 47.

10. Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. Endocrinology. 2000; 141: 1317–1324.

11. Viganò P, Lattuada D, Mangioni S, Ermellino L, Vignali M, Caporizzo E, et al. Cycling and early pregnant endometrium as a site of regulated expression of the vitamin D system. J Mol Endocrinol. 2006; 36: 415–424.

12. Fransasi JM, Molinaro TA, Dubell EL, Scott KL, Ruiz AR, Forman EJ, et al. Vitamin D levels do not affect IVF outcomes following the transfer of euploid blastocysts. Am J Obstet Gynecol. 2015; 212(3): 315. e1-6.

13. Paffen O, Ferrini S, Viganò P, Pagliardini L, Papaleo E, Candidi M, et al. Vitamin D deficiency and infertility: insights from in vitro fertilization cycles. J Clin Endocrinol Metab. 2014; 99(13): 2372-2376

14. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. J Postgrad Med. 2002; 48(3): 206-208.

15. Farrar MD, Zulf Mughal N, Adams JE, Wilkinson J, Berry JI, Edwards L, et al. Sun exposure behavior, seasonal vitamin D deficiency and relationship to bone health in adolescents. J Clin Endocrinol Metab. 2016; 101(8): 3105-3113

16. Eloï M, Horvath DV, Szefnfeld VL, Ortega JC, Rocha DA, Szefnfeld J, et al. Vitamin D deficiency and seasonal variation over the years in São Paulo, Brazil. Osteoporos Int. 2016; 27(12): 3449-3456.

17. Turkish State Meteorological Service. www.mgm.gov.tr. Official statistics of Ankara. Available from: www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler/istatistik.aspx?m=ANKARA#sIB [last accessed on 16 June 2017].

18. Anifandis GM, Dafopoulos K, Messini CI, Chalvatzas N, Iliakos N, Pournaras S, et al. Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. Reprod Biol Endocrinol. 2010; 8: 91.

19. Luk J, Torrealday S, Neal Perry G, Pal L. Relevance of vitamin D in reproduction. Hum Reprod. 2012; 27(10): 3015-3027.

20. Hekimsoy Z, Dinç¸er G, Kafesçi¸ler S, Onur E, Güvenç Y, Pala T, et al. Vitamin D status among adults in the Aegean region of Turkey. MC Public Health 2010; 10: 782

21. Aydogmus S, Kelekci S, Aydogmus H, Eriş S, Desdicioğlu R, Yılmaz B, et al. High prevalence of vitamin D deficiency among pregnant women in a Turkish population and impact on perinatal outcomes. J Matern Fetal Neonatal Med. 2015; 28: 1828-1832.

22. Soria M, Pradillo G, García J, Ramón P, Castillo A, Jordana C, et al. Pregnancy predictors after intrauterine insemination: analysis of 3012 cycles in 1201 couples. J Reprod Infertil. 2012; 13: 158–166.

23. Ott J, Wattler L, Kurz C, Seemann R, Huber JC, Mayerhofer K, et al. Parameters for calcium metabolism in women with polycystic ovary syndrome who undergo clomiphene citrate stimulation: a prospective cohort study. Eur J Endocrinol. 2012; 166: 897-902.

24. Colonese F, Laganà AS, Colonese E, Sofo V, Salmeri FM, Granese R, et al. The pleiotropic effects of vitamin D in gynaecological and obstetric diseases: an overview on a hot topic. Biomed Res Int. 2015; 2015: 986281.

25. Vilarino FL, Bianco B, Lerner TG, Teles JS, Mafra FA, Christofolini DM, et al. Analysis of vitamin D receptor gene polymorphisms in women with and without endometriosis. Hum Immunol. 2011; 72(4): 359-363.

26. Nuouja-Hultunen S, Tomas C, Bloigu R, Tuomivaara L, Martikainen H. Intrauterine insemination treatment in subfertility: an analysis of factors affecting outcome. Hum Reprod. 1999; 14: 698–703.

27. Halloran BP, DeLuca HF. Effect of vitamin D deficiency on fertility and reproductive capacity in the female rat. J Nutr. 1980; 110(8): 1573–1580.

28. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, et al. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet. 1997; 16(4): 391-396.

29. Panda DK, Miao D, Tremblay ML, Sirios J, Farookhi R, Hendy GN, et al. Targeted ablation of the 25-hydroxyvitamin D 1alpha -hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. Proc Natl Acad Sci USA. 2001; 98(13): 7498-7503.

30. Jukic AM, Steiner AZ, Baird DD. Association between serum 25-hydroxyvitamin D and ovarian reserve in premenopausal women. J Clin Endocrinol Metab. 2007; 92(10): 3590-3594.

31. Carlotto FD. Vitamin D improves endometrial thickness in PCOS women who need intrauterine insemination: a randomized double-blind placebo-controlled trial. Arch Gynecol Obstet. 2014; 289(4): 865-870.

32. Ozkan S, Jindal S, Greenseid K, Shu J, Zeitlian G, Hickmon C, et al. Replete vitamin D stores predict reproductive success following in vitro fertilization. Fertil Steril. 2010; 94(4): 1314–1319.