Original article

Vitamin D deficiency in Infertility cases, a pilot study in Delhi

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Abstract:

Background: Vitamin D deficiency is associated with infertility, decreased pregnancy rates, and hormonal changes. Asians appear to have a reverse correlation with pregnancy rates and vitamin D levels. There are some conflicting studies regarding vitamin D levels in relationship to infertility.

Objective: To analyze Vitamin D in infertile women coming to hospital for treatment and compare with healthy fertile controls. To study if there is any correlation of Vitamin D with TSH (thyroid stimulating hormone), FSH (follicle stimulating hormone), LH (luteinizing hormone) and Prolactin levels in these patients. Our study also tries to delve into the question if there is a need to do Vitamin D levels in infertility cases routinely.

Methods: The study was conducted in department of Biochemistry and Gynecology of tertiary care hospital of Delhi after obtaining approval from institution's Ethical committee. Blood samples were taken from infertile women (n=58), age group 20-35 years, with both (84%) primary and (16%) secondary infertility coming to Gynecology OPD for their treatment. Age matched healthy females (n=30) without any history of infertility, were enrolled as controls in the study. Consent was obtained from all participants. Samples were analyzed for Vitamin D, TSH, Prolactin, FSH and LH.

Result: Infertile women had significantly lower vitamin D levels (p value<0.001) and higher TSH (p value<0.001), higher LH levels (p value<0.05) and higher FSH (p value<0.05) values as compared to healthy volunteer controls of same age and gender. The levels of Prolactin were higher in cases than controls, though the difference was not significant (p value >0.05).

Pearson's correlation coefficient calculation in the cases (infertile women) showed no significant association of Vitamin D with TSH, FSH, LH and Prolactin (p value >0.05).

Vitamin D deficiency (< 50nmol/l) was present in 67% of total study population (n=88). 91.4% of cases (n=58) were vitamin D deficient. The odds ratio for vitamin D deficiency in infertile female was found to be 42.4(95% CI: 11.7-152.6, p value<0.001).

Conclusion: Vitamin D deficiency was prevalent in 91.4% of infertile women of the study group; however the deranged hormonal profile in infertile cases did not have any significant correlation with Vitamin D levels. Mechanism by which Vitamin D results in adverse fertility out comes in humans needs to be elucidated by further studies.

Key words: Vitamin D deficiency,TSH, FSH, LH,Prolactin, Infertility

Introduction:

The non-skeletal effects of vitamin D have been the focus of much interest in the past decade and an accruing body of literature is supportive of relevance of vitamin D for a variety of organ systems beyond the skeleton [1, 2].

Vitamin D has recently received attention for the role it plays in reproduction and fertility. Vitamin D receptors (VDR) facilitate the biological activity of

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vitamin D and are found in many tissues such as human testis, sperm, epididymis, seminal vesicle, prostate, ovaries, uterus, placenta, cervix, breast tissue, the pituitary, and hypothalamus[3-5]. Vitamin D insufficiency or deficiency is present in 58% to 91% of women with infertility[6-8]. Some risk factors for vitamin D deficiency in women include an elevated body mass index (BMI), polycystic ovarian syndrome (PCOS), Asian ethnicity, and those of black ethnicity[7]. Vitamin D is also involved in the regulation of several hormones in the body including the anti-Müllerian hormone (AMH), follicle stimulating hormone, estradiol (a type of estrogen), and progesterone, all having to do with fertility[5]. Many studies show higher pregnancy rates, better IVF outcome with better vitamin D status[8-10]; and there have been studies from Iran[11] and Greece[12] that show no significant correlation between pregnancy rate and serum vitamin D levels .We aim to find the vitamin D status in infertile women coming to tertiary care hospital in New Delhi and to study correlation with deranged fertility parameters in them.

MATERIALS & METHODS

This observational, case control study was carried out in women in the age group 25–40 years, presenting for hormonal evaluation for treatment of infertility in the hormone lab of a tertiary care hospital in Delhi. Infertility was defined as couple that has never conceived despite exposure to the risk of pregnancy for a period of 1 year.

The inclusion criteria for the selection of cases were diagnosis of primary infertility, age between 20-40 years and duration of marriage more than one year. The exclusion criteria that were adopted during case selection were male factor infertility and amongst the female factors were tubal factor, any congenital anomaly of the urogenital tract, or any obvious organic lesion. Fifty eight consecutive subjects with both primary (84%) and secondary (16%) infertility were enrolled in the study after a written and informed consent. Control group consisted of healthy female volunteers of comparable age group; 25-38 years, with no history of infertility (n=30).All the controls had at least one child. All the women reported for hormone analysis on the second or third day of menstrual cycle. Fasting samples were drawn for analysis of LFT, KFT, blood sugar, LH, FSH, Prolactin, TSH and Vitamin D.

Assays

Blood glucose levels were estimated using glucose-oxidase (GOD-POD) method [13], Serum urea was estimated using urease-GLDH kinetic method [14, 15] and serum creatinine levels were measured using modified jaffe's kinetic method [16]. Liver enzymes ALT, AST and ALP were measured using IFCC recommended enzymatic methods [17-19]. All the mentioned biochemical tests were done using automated analyzer (AU480, Beckman Coulter). Serum hTSH (highly sensitive TSH) [20, 21], FSH [22], LH [23] and Prolactin [24] levels were estimated immediately using chemiluminescence based immunoassay (Beckman Access II, Beckman Coulter, Inc., Fullerton, CA).

FSH and LH assay both had a sensitivity of 0.2mIU/ml and an inter assay coefficient of variation (CV) of less than 10%.

hTSH had sensitivity of 0.003 µIU/L and inter assay CV of less than 20%.

Prolactin assay had analytical sensitivity of 0.25ng/ml and less than 10% CV.

Serum 25(OH) Vitamin D levels were assayed using ELISA (DRG kit, The USA) with a sensitivity of 1.5 ng/ml and an inter assay (CV) of less than 10.5%. All

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the analysis was performed in duplicates and average values used.

Statistical analyses

All analysis was done using IBM SPSS software (Version 20.0, IBM SPSS, IL, USA). Group data are presented as mean values ± S.D. Quantitative data was assessed using independent sample student's t-test. An association between study variables was assessed using Pearson's correlation analysis and odds ratio calculation. A value of $p \leq 0.05$ was considered statistically significant.

Results

The average age of women in the case group was 31.2± 3.7 years, and the control group was 30.2± 3.8 years (p value> 0.05). The BMI in cases was 24.56± 0.62 m/kg^2 and controls was 23.17± 0.81 m/kg^2 (p value >0.05).

Vitamin D deficiency is defined as serum value < 50 nmol/l, insufficiency as 50 to 75nmol/l and sufficiency as >75nmol/l. Infertile women had significantly lower vitamin D levels (p value<0.001) as compared to controls (Table1).Vitamin D deficiency was seen in 91.4% of cases and 20% of controls (Table 2). The odds of Infertility in Vitamin D deficiency are 42.4 (95% CI: 11.7-152.6, p value<0.001). Although the odds ratio is very high, the confidence interval is very wide; hence it is not a very precise estimate. Infertile women also had higher TSH (p value<0.001), higher LH levels (p value<0.05) and higher FSH levels (p value<0.05).The levels of Prolactin are higher in cases than in controls, though the difference is not significant (p value >0.05) [Table 1].

Pearson's coefficient showed no significant correlation of Vitamin D among infertile cases with TSH, LH, FSH and Prolactin (p value >0.05) in the present study. Only serum FSH was significantly correlated with serum LH values in cases (r=0.676, p value < 0.001).

Tables 1: Shows the mean values along with standard deviation of various parameters studied in infertile women and their controls. The p value (calculated by independent T test) is considered significant if <0.05.

	Normal range	Cases(n=58) Mean ±std. dev.	Controls (n=30) Mean± Std.dev	P value
Vitamin D nmol/l	<50:deficiency 50-74:insufficient 75-250:sufficient >250:toxicity	21.1± 18.0	64.3±20.3	0.000
TSH µIU/ml	0.4-4.2	7.8±6.1	3.4±1.1	0.000
FSH mIU/ml	3.1-7.9 (follicular phase)	24.8±35.3	12.4±4.9	0.003
LH mIU/ml	1-18 (follicular phase)	18.3±21.4	8.9±4.5	0.071
Prolactin ng/ml	2-29	18.2±11.5	16.9±13.8	0.674

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Table 2: Shows vitamin D distribution in cases (infertile females) and controls (healthy and fertile females).

Vitamin D levels in nmol/l		Cases (n=58)	Controls (n=30)
<50	Deficient	91.4%	20%
50-74	Insufficient	6.9%	30%
75-250	Sufficient	1.7%	50%
>250	Toxicity	-	-

Discussion

Statistics suggest that among infertile women there is a high incidence of Vitamin D deficiency. Li and colleagues[7] found that 90.8% of women being worked up for infertility had insufficient (68.6% <32ng/mL) or deficient (22.2% <20ng/mL) vitamin D levels. This study was conducted in San Francisco, USA and they concluded that Asian and Black women have lower Vitamin D levels compared to Caucasians. Also the Vitamin D levels in 1182 infertile women did not correlate with ovarian reserve, measured by FSH and AMH (Anti Mullerian Hormone). Likewise, Ozkan and colleagues [8]from Montefiore medical Centre, USA, found in a population of infertile women 63% had vitamin D levels that were insufficient (36% 20-30ng/mL) or deficient (27% <20ng/mL). In addition, Anifandis and colleagues[12] from Greece reported 79%of women undergoing in vitro fertilization (IVF) were vitamin D insufficient (48% 20.1-30ng/mL) or deficient (31% <20ng/mL). Our study reports 91.4% infertile women having vitamin D deficiency. The high prevalence could be attributed to race, ethnicity, cultural practices and level of malnutrition that exists in population coming to government hospital in North India. The controls used in the study were healthy volunteers; they were working staff of the hospital and were not patients.

There have been studies in the past showing correlation of Vitamin D with TSH and FSH; incidentally these studies were not done in infertile women. A study by AMH.Mackawy et al[25] shows a significant correlation between vitamin D deficiency and degree and severity of hypothyroidism. A study by AMZ Jukic et al[26] suggested that vitamin D may influence the ovarian reserve. The study showed inverse relation vitamin D and FSH levels in 1430 peri-menopausal women.

There has been controversy regarding beneficial effects of Vitamin D in fertilization and pregnancy. Study by Anifandis et al [12], from Greece (n=101) reported pregnancy rates were lower with high Vitamin D (p value <0.05).

Study by Aleyasin et al [27], from Iran (n=82) reported that Vitamin D deficiency doesn't play pivotal role in outcome of ART (artificial reproductive technique).

Similarly a study by Firouzabadi et al [11], from Iran (n=221) reported no significant correlation between pregnancy rate and serum Vitamin D levels.

In the present study it is difficult to explain lack of correlation between Vitamin D and deranged fertility parameters in the cases due to small sample size. It can be taken as pointing towards some mechanism other than hormonal by which lack of vitamin D affects ability to conceive.

There is some evidence that vitamin D deficiency and its effects on fertility may be indirect. Without vitamin D, the body absorbs up to 30% less calcium and 20% less phosphorus. In experimental conditions, when the hypocalcaemia and

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hypophosphatemia were corrected in the female mice, their fertility returned[28]. It's possible the primary cause of infertility may be hypocalcaemia and/or hypophosphatemia. But these studies have only been done in animals.

The reports of Vitamin D deficiency affecting fertility have not been very conclusive especially in Asian population. More advanced research is required in this regard with larger sample size before we recommend it to be included in the workup of infertile women of this country. Nevertheless, Vitamin D is a relatively safe and inexpensive supplement. Sufficient vitamin D levels could possibly increase a couple's overall health and hence likelihood of pregnancy.

Conclusion

A growing body of literature suggests that an individual's vitamin D status may adversely impact reproductive functions. However, there is dearth of prospective interventional studies and studies that define the mechanisms whereby vitamin D affects reproductive physiology in humans. Although in present study we see that infertile patients have significantly low vitamin D, there was no significant correlation between Vitamin D and the deranged hormone profile. The draw- back of study is small sample size .We may conclude that vitamin D deficiency is highly prevalent (91.4%) in infertile women in present study.

References

1. Walters MR. Newly identified actions of the vitamin D endocrine system. Endocr Rev. 1992; 13:719–763.
2. Reichel H, Koeffler HP, Norman AW. The role of vitamin D endocrine system in health and disease. N Engl J Med. 1989; 320:980–991.
3. Martin Blomberg Jensen, John E. Nielsen,Anne Jørgensen, Ewa Rajpert-De Meyts,David Møbjerg Kristensen, Niels ørgensen,, et al. Vitamin D receptor and vitamin Dmetabolizing enzymes are expressed in the human male reproductive tract. HumanReproduction. 2010;25(5):1303-1311
4. Corbett ST, Hill O, Nangia AK. Vitamin D receptor found in human sperm. Urology. 2006;68(6):1345-1349.
5. Irani M, Merhi Z. Role of vitamin D in ovarian physiology and its implication inreproduction: A systematic review. Fertility & Sterility. 2014;102(2):460-468.
6. Rudick B, Ingles S, Chung K, Stanczyk F, Paulson R, Bendikson K. Characterizing theinfluence of vitamin D levels on IVF outcomes. Hum Reprod. 2012;27(11):3321-3327.
7. Li L, Schriock E, Dougall K, Givens C. Prevalence and Risk Factors of Vitamin D Deficiency in Women With Infertility Fertility and sterility 2012;97(3)
8. Ozkan S, Jindal S, Greenseid K, et al. Replete vitamin D stores predict reproductive success following in vitro fertilization. Fertil Steril. 2010;94(4):1314-1319.
9. Garbedian K, Boggild M, Moody J, Liu KE. Effect of vitamin D status on clinical pregnancy rates following in vitro fertilization. CMAJ Open. 2013;1(2):E77-E82.

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10. Alessio Paffoni, Stefania Ferrari, Paola Viganò, Luca Pagliardini, Enrico Papaleo, Massimo Candiani, et al. Vitamin D deficiency and infertility: Insights from invitro fertilization cycles. J Clin Endocrinol Metab. 2014;99(11):E2372-E2376.

11. Firouzabadi R, Rahmani E, Rahsepar M, Firouzabadi M. Value of follicular fluid vitamin D in predicting the pregnancy rate in an IVF program. Arch Gynecol Obstet.. 2014;289(1):201-206.

12. Anifandis GM, Dafopoulos K, Messini CI, Chalvatzas N, Liakos N, Pournaras S, et al.Reprod Biol Endocrinol. 2010 Jul 28;8:91-95.

13. Thomas L. Blood glucose. In:Thomas L, ed. Clinical laboratory diagnostics. Use and assessment of clinical laboratory results. Frankfurt/Main: TH-Books Verlagsgesellschaft, 1998:131-37.

14. Talke, H. and Schubert, G.E., Klinische Wochenschrift. 1965; 43: 174.

15. Manoukian, E. and Fawaz, G.Z.,.Klin Chem Klin Biochem. 1969; 7: 32,

16. Cook, J.G.H., Clin Chem Acta. 1971; 32: 485.

17. Saris, N.E., Clin Chem. 1987; 24: 720 – 721.

18. Bergmeyer, H.U. and Horden, M., J. Clin Chem Clin Biochem. 1980; 18: 521-524.

19. Tietz, N.W.(ed), Textbook of Clinical Chemistry, W.B. Saunders, 1986.

20. Spencer CA, Takeuchi M, Kazarosyan M, MacKenzie F, Beckett GJ, Wilkinson E. Interlaboratory/intermethod differences in functional sensitivity of immunometric assays of Thyrotropin (TSH) and impact on reliability of measurement of subnormal concentrations of TSH. Clin Chem 1995; 41/3, 367-374.

21. Alexander Jr. RL. The diagnostic importance of third-generaton methods for the assay of thyrotropin (TSH). American Clinical Laboratory, 18, December 1994 - January 1995.

22. Adashi EY. The ovarian life cycle. In Reproductive Endocrinology. Edited by Yen SSC and Jaffe RB. Philadelphia, PA: WB Saunders Co., 1992; 22: 1-28.

23. Carr BR. Disorders of the ovary and female reproductive tract. In Williams Textbook of Endocrinology, 8th ed. Edited by Wilson JD and Foster DW. Philadelphia, PA: WB Saunders Co., 1992; 733-798.

24. Kronenberg HM, Melmed S, Polonsky KS, Larsen PR. Williams textbook of endocrinology, 11th ed. Philadelphia, PA: Saunders Elsevier, 2008; 542.

25. Dr Amal Mohammed Husein Mackawy, Bushra Mohammed Al-ayed, Bashayer Mater Al-rashidi.Vitamin D deficiency and its association with thyroid disease. Int J Health Sci.2013; 7:267-275.

26. Anne Marie Z Jukic, Anne Z Steiner, Donna D Baird. Association between serum 25-hydroxy vitamin D and ovarian reserve in premenopausal women. Menopause.2015; 22:312-316.

27. Aleyasin A, Hosseini MA, Mahdavi A, Safdarian L, Fallahi P, Mohajeri MRet al. Predictive value of the level of vitamin D infollicular fluid on the outcome of assisted reproductive technology. Eur J Obstet GynecolReprod Biol. 2011;159(1):132-137.

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

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