Short Communication

Vitamin D and Vitamin D Receptor in Female Infertility

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ABSTRACT

Objective: To investigate the possible association of serum and follicular fluids(FF) Vitamin D levels with reproductive outcomes after assisted reproductive techniques (ART). Secondly, to detect Vitamin D Receptors (VDR) in serum and follicular fluid (FF) of ovarian follicle and explore its relationship with Vitamin D levels as well as reproductive outcomes.

Methodology: This study was conducted on May 2019 in Aga Khan Hospital, Karachi, Pakistan. A total of 8 infertile female subjects were conveniently recruited. Serum was collected and follicular fluid(FF) was obtained by transvaginal-guided follicular puncture; 34 hours after intramuscular administration of Human Chorionic Gonadotrophin (HCG). Vitamin D and VDR was assessed in serum as well as in FF by Paired Sample t-test.

Results: Females 2 out of 8 acquired clinical pregnancy, whereas 3 had pre-clinical abortion or no pregnancy. Vitamin D and VDR were detected highest in those female patients that acquired pregnancy in comparison to the pre-clinical abortion or non-pregnant ones (p value<0.007). Serum VDR did not show any significant results in all groups

Conclusion: Vitamin D in serum and FF and vitamin D receptor in serum and FF were associated with number of oocytes. High levels of VDR in FF observed in clinical pregnancy group proved our hypothesis that the presence of VDR in FF is a contributing factor for successful conception after ART success.

KEYWORDS: Assisted Reproductive Technique, female infertility, follicular fluid, Vitamin D, Vitamin D receptor

INTRODUCTION

Vitamin D (VD) is a known fat solubilizing vitamin, playing a pivotal role in the homeostasis of phosphorus and calcium, thus maintaining healthy teeth and bones. VD also fortifies the body against ailments such as; Diabetes, obesity, cancer, cardiovascular disease and even in infertility.[1] Vitamin D deficiency (VDD) is also observed to be associated with many female reproductive maladies including. Endometriosis, polycystic ovarian syndrome (PCOS), gestational diabetes, and bacterial vaginosis etc.[2] According to a study in Oklahoma, VDD is highly seen in women who are diagnosed with PCOS.[3] Another study in Pakistan, have also reported the prevalence of VDD in women with PCOs.[4] Due to the role of VD seen in the process of steroidogenesis and in the activity of aromatase, it can be a possibility that VD effects the quantity and quality of oocytes and hence the pregnancy by increasing the production of Estradiol.[5,6] In a study conducted in California, 1192 women of reproductive ages were recruited and among them it was observed that the infertile women had low VD levels; 68.6% had <32 ng/ml whereas, 22.2% had <20 ng/ml levels.[7]

Vitamin D receptor (VDR) is the transcriptional protein located in the target cell's nucleus and various tissues (uterus, endometrium, ovaries and placenta) and helps in the biological actions of VD.[8,9] After VD binds with its receptor, it regulates Homeobox protein Hox-A10 (*HOXA10)* gene activity, immune response of endometrium and reduces T-cell activity.[10,11]

According to previous a study, it was observed that females having high levels of VD in follicular fluid (FF) and serum have a higher chance of conceiving following IVF, hence VD levels in FF independently can determine IVF outcome.[12] With this background, we came across with following research questions; Is there a role of FF Vitamin D with pregnancy

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outcome? What is the importance of VDR in acquiring pregnancy outcome? So, the objective of our study was to find an association between FF and serum Vitamin D and VDR, with pregnancy outcome after in vitro fertilization.

METHODOLOGY

This study was conducted in Aga Khan Hospital, Karachi Pakistan on May 2019, after acquiring approval from the institutional ethical review committee(AKU-BBS-2019-0557-5582).Using convenient sampling methodology, eight infertile females with unexplained cause of infertility undergoing Assisted Reproductive Technique (ART) were recruited for this study after obtaining a written informed consent, they were expected to fulfill the criteria of primary infertility, and were between the ages of 18-40 years from Infertility Medical Centre, Karachi, Pakistan. Women more than 40 years, having BMI more than 30 kg/m^2, on oral contraceptive pills and hormonal treatments or practicing any contraceptive measures and women with serious general health status was excluded. Their ovaries were stimulated by exogenous gonadotropin (follicle stimulating hormone; FSH) from Day 5 of the cycle and then given human chorionic gonadotropin (100,000) injections when at least 2-3 follicles reached maturity (a diameter of 18 mm). Transvaginal-guided follicular puncture and aspiration of FF was performed under general anesthesia 35 ± 1 hour after administration of HCG. Both serum and FF samples were collected on the same day, after centrifuge at the speed of 3000xg for 15 minutes, supernatant was collected and stored at -80^{o}C. VDR levels in serum and FF were observed by using commercially available Enzyme Linked Immunosorbent Assay Kit (Cat. No: SEA475Hu, Cloud-Clone Corp) with a detection range of 0.625 – 40 ng/ml. The analytical sensitivity was less than 0.225 ng/mL, and intra-inter assay coefficient of variation (CV) was <10% and 12% respectively. Whereas, VD levels in serum and FF observed by Human, 1,25-Dihyroxy Vitamin D ELISA Kit (Cat#95503), with an intra-inter assay coefficient of variation (CV) of 2.7% and 4.3%, respectively. The lowest limit of detection was 2.8 ng/ml.

Statistical analysis: Data was analyzed using IBM SPSS Version 23. The distribution of quantitative variables was computed by their means and standard error of mean (SE) and assessed by Spearman's Rank Correlation Test. Paired Sample t-test was used to compare means. Statistical significance was set at $p<0.05$.

RESULTS

A total of 8 infertile female subjected were recruited for this study. Their Demographic and Biochemical data are mentioned in Table 1.

Table 1: Demographic, Biochemical Characteristics of infertile females (N=8)

Factors	Mean ± SE
Age (years)	34.38 ± 1.73
BMI (kg/m^2)	21.38 ± 0.86
Serum Vitamin D (ng/ml)	28.45 ± 1.27
Vitamin D Follicular Fluid (ng/ml)	29.84± 1.26
VDR in follicular fluid (ng/ml)	27.32 ± 7.48
Serum VDR (ng/ml)	0.1 ± 0.09

Table 2: Correlation of serum and follicular fluid Vitamin D with study parameters

Factors	Correlation Coefficient/ r value	p-value
Serum VD with VD in FF	0.972	0.001*
Serum VD with serum VDR	0.713	0.047*
Serum VD with FF VDR	0.959	0.0001*
VD in FF with serum VDR	0.586	0.126
VD in FF with FF VDR	0.947	0.001 *
serum VD with Oocytes	0.857	0.007*
serum VDR with Oocytes	0.488	0.220
FF VD with Oocytes	0.890	0.003*
FF VDR with Oocytes	0.883	0.004 *
Serum VD with BMI	-0.690	0.05 *
FF VD with BMI	-0.689	0.05*
serum VDR with BMI	-0.723	0.043*
FF VDR with BMI	-0.764	0.027*
FF VDR with VD	0.571	0.139

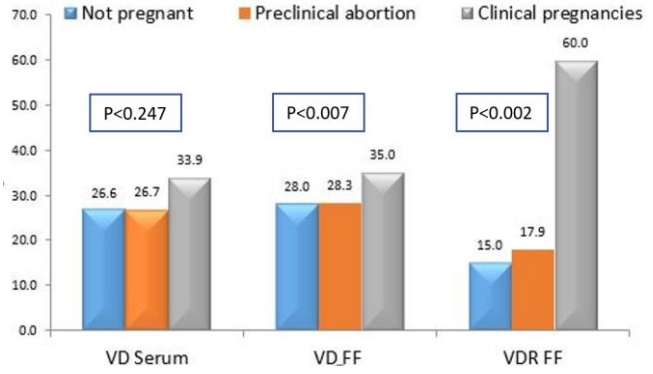
*Significant p-value of ≤0.05 via Spearman's Rank Correlation Test

Table 2 represents, correlation in Serum and FF levels of VD and VDR with study parameters. A positive significant correlation was observed in serum VD with different parameters such as VD in FF, serum VDR, VDR in FF and number of oocytes. A negative correlation of BMI with VD and VDR in both serum and FF was observed (Table 2).

Out of the 8 patients, 2 females acquired clinical pregnancy (25%), 3 female patients (37.5%) had pre-clinical abortion and 3 did not get pregnant. Those females who conceived had the highest VD in FF (p value<0.002) levels as compared to the pre-clinical abortion and non-pregnant females (p value<0.247). VDR in FF were highest in clinical pregnancy (p

value<0.002). However, Serum VDR did not show any significant results in all the groups (Figure 1).

Figure 1 demonstrates the detected levels of Vitamin D in serum, Vitamin D in follicular fluid and Vitamin D Receptor in follicular fluid of infertile female undergoing Assisted Reproductive Technique (n=8) subjects



*Significant at p value ≤0.05 by Paired Sample t-test

DISCUSSION

We observed high vitamin D levels in serum and FF of females with successful conceptions which is supported by our previous study, in which we documented that vitamin D helped in acquiring optimum endometrial thickness necessary for successful implantation during ICS.[13] and also in another study, higher serum and FF level of vitamin D was found concomitant with better pregnancy rates after intracytoplasmic sperm injection(ICSI).[14] Furthermore, Farzadi et al. observed that vitamin D though did not affect number and quality of oocytes yet improved success rate after IVF[(11)]. Vitamin D levels in FF showed no correlation with oocyte quality, fertilization rate, and reproductive outcome.[15] A number of biological function of vitamin D is facilitated by VDRL.[16]Our findings showed a significant relation between vitamin D and VDR. Although, VDR is present in follicular fluid but its association with Vitamin D may not be functional which give rise to infertility in the females. In a previous animal study, the role of VDR has been reported through the effect on availability of calcium ions in null mutant female mice.[17] Current study major finding was the positive significance relation between VDR in serum collected from blood and VDR in FF of infertile females indicating that increase of VDR in serum increases significantly in follicular fluid.

A negative significant relation between BMI and vitamin D and VDR levels of the subjects indicates that as their BMI increases their VD and VDR levels significantly decreases. Significant differences was observed in VDR and vitamin D of FF in a study with females having Polycystic Ovarian Syndrome and overweight patients.[18] In order to improve Vitamin D and VDR, infertile females should be asked to reduce their BMI as this will help in increasing VDR and Vitamin D in FF and hence chance of conception. Hence, predominance of vitamin D in serum and FF helped infertile females in acquiring clinical pregnancy after ICSI. However, negative levels of serum VDR indicates that vitamin D receptors cannot be found in serum, but can be detected in the follicular fluid playing its role in assisting vitamin D of infertile females to achieve their pregnancy, this novel approach of detecting VDR in follicular fluid is taken for the first time in our study. Nonetheless, proper randomized clinical trials with larger sample size are required to accomplish more convincing results about the auspicious role of vitamin D and VDR in the management of female assisted reproduction.

Limitations: We did not calculate sample size and conducted our pilot study on a very small sample.

CONCLUSION

Vitamin D in serum and FF and vitamin D receptor in serum and FF were associated with number of oocytes. High levels of VDR in FF observed in clinical pregnancy group proved our hypothesis that the presence of VDR in FF is a contributing factor for successful conception after ART success.

Conflict of Interest: *None*

Funding Source: *None*

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Author's Contribution:

Nida Farooqui	Data collection, literature search, draft writeup.
Qurat-ul-ain Maha,	Literature search, manuscript write up and approved it
Mussarat Ashraf	Study concept and design, analysis, integration of results, result writeup
Arfa Azhar	Data Collection Statistical analysis proved the manuscript.
Farrukh Jehan	Concept, Data analysis & approved the manuscript.
Dr. Rehana Rehman	Concept, study design, result write up & approved manuscript.
	All authors are equally accountable for research work and integrity

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