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Effect of Vitamin D Status on Clinical Pregnancy Rate in Case of Intracytoplasmic Sperm Injection (ICSI)

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Abstract

Background: Vitamin D had been suggested to have a role in human reproduction. We aimed to investigate if vitamin D levels can predict implantation and clinical pregnancy rates in infertile females after Intra-cytoplasmic sperm injection (ICSI).

Methods: Vitamin D levels had been evaluated prospectively by levels of serum 25-hydroxy-vitamin D (25[OH] D) levels, in a cohort of one hundred and fifty couples undergoing ICSI. Serum samples had been collected one week prior to oocyte retrieval. Patients had been classified according to vitamin D serum level into sufficient (≥ 75 nmol/L) or insufficient (<75 nmol/L).

Results: 38.97% of included females had sufficient vitamin D levels mean while 61.03% had insufficient vitamin D levels. Females with sufficient levels of vitamin D had a higher clinical pregnancy rates per ICSI cycle (58.49%) in comparison to those with insufficient levels (36.14%; p 0.011).

Introduction

Incidence of infertility approximately more than 15% of all couples trying to conceive. Vitamin D role in human reproduction and vitamin D level in prediction of reproduction success rates after ICSI had been supported by recent studies [1,2].

Vitamin D and Infertility

Vitamin D is a pro-hormone that either synthetized in the skin endogenously or obtained from the diet exogenously. Vitamin D is metabolized primarily in the hepatocytes to (25[OH]D), the serum level of (25[OH]D) which can be used as an indicator of vitamin D status [3].

Vitamin D is recently investigating the relation between it and fertility so; there is no specific cut-off levels had been referenced in the literature [4].

While the guideline of Canada defined the Vitamin D deficiency level less than or equal 25 nmol/L. Insufficiency Vitamin D levels between 25-74 nmol/L, and sufficiency Vitamin D levels of more than or equal 75 nmol/L [5]. There is relation between lower level of vitamin D and higher incidence of different types of cancer and impaired immune response [6,7]. Lower level of vitamin is highly prevalent in females of reproductive age. There is study reported that about 25% of black and about 15% of white females of reproductive age had been insufficient vitamin D levels [8]. In previous study had been reported that a 36% of vitamin D insufficiency (50-74 nmol/L) and a 27% of deficiency (<50 nmol/L) among females of reproductive age with infertility [9]. The vitamin D insufficiency (50-75 nmol/L) or deficiency (<50 nmol/L) was 79% in a population of females undergoing ART [10].

There are vitamin D receptors found in different reproductive organs such as ovary and uterus and many studies had been investigate the relation between vitamin D level and success rate following ICSI in human [11].

A cohort prospective study that measured the level of 25-hydroxy-vitamin D in the FF of 84 females undergoing ICSI found that females with increased levels of 25-hydroxy-vitamin D in their FF were significantly increase rate of implantation and pregnancy following ICSI [12-15].

In contrast to a small prospective study that found no significant difference in biochemical or clinical pregnancy rates across the level of vitamin D in follicular fluid [11]. There is a cohort prospective study that suggested higher levels of 25-hydroxy-vitamin D, in combination with decreased levels of glucose, in follicular fluid may have a negative effect on the ICSI success rates [16,17]. In our study, we aimed to determine whether levels of vitamin D are predictive of ICSI outcomes among infertile females [13].

Subjects and Methods

This study was conducted at the International Islamic Centre for Population Studies and Research (IICPSR)-ART unit, Al-Azhar University, Cairo, Egypt. During period of six months interval. This study included 150 couples, and 136 were included in our analysis. We excluded 14 couples because they did not complete the cycle due to negative TESE result.

Informed written consent was obtained from all included couples and study was depended on the institution's research ethics board. All included females were classified according to vitamin D serum level into two groups:

1) Group I (sufficient ≥ 75 nmol/L 25-OH vitamin D3).

2) Group II (insufficient <75 nmol/L 25-OH vitamin D3).

Inclusion Criteria

Age of female ranges between 20-35 years old, Follicle-stimulating hormone level 12 IU/L or lower, all patients have the same cause of infertility (male factor), all patients undergoing ovulation induction using a long protocol, all patients will be receiving the same drugs of down regulation and gonadotrophins hormones and embryo transfer will be in the third day after ICSI.

Exclusion Criteria

Patients under vitamin D therapy, renal patients, patients with parathormone gland defects and GIT disease e.g., protein enteropathy.

All included couples were subjected to the following

- **Full history taking include obstetrical and gynecological history:** Age, weight, height, parity, occupation, special habits and duration of infertility.
- **Complete physical examination:** Vital data and full general examination and pelvic assessment.
- **Assessment of male partner:** Ejaculated sperm evaluation or testicular Sperm evaluation.
- All females were subjected to:

All females' partners underwent ovulation induction using a long protocol. The long protocols used long acting GnRH analogue is given in the midluteal phase i.e., day 21 of the cycle, then gonadotrophins therapy was started on the second day of menstruation after E_2(Estradiol) level less than 50 pg/mL and the dose of gonadotrophins adjusted according to patient age, basal hormone and Antral follicle count (AFC). Oocyte-cumulus complexes (OCC) were recovered 36 h after the administration of 5000 or 10000 IU of human chronic gonadotrophin (HCG).

- Venous blood sample was withdrawn within 1 week before Oocytes retrieval to measure of 25-OH vitamin D_3 levels according to vitamin D assay.

Intra-Cytoplasmic Sperm Injection (ICSI)

A-Samples preparation: Sperm samples for ICSI (ejaculated and testicular) were done according to the methods described earlier then a small amount of 10% Polyvinyl Pyrolidone (PVP) warmed at 37°C and put the selected sperms in it.

B-Oocyte collection, identification: Oocyte retrieval was performed 36 hours after the Human Chronic Gonadotropin (HCG) by trans-vaginal ultrasound-guided needle aspiration under general anesthesia. Follicular fluid was aspirated into sterile tubes. The oocyte-cumulus were identified and washed in BM1 (Bulletined DE control milieu) Menezo Media, (Euro bio) and equilibrated at 37°C in 5% CO_2, then washed and placed into four well (nunc) dishes containing the same medium and incubated at 37°C in 5% CO_2 for approximately 1/2 hour.

C-Denudation: The oocyte placed in a 100 µ 1 drop of buffered containing Hualu-ronidase 80 IU/ml (Sigma, USA) 30-45 seconds, and then the oocyte was removed and placed in 100 µl drop of BM1 medium. The corona cells were removed by gentle aspiration of the oocyte in and out of a sterile drawn pipette. When denudation was completed, the oocyte was washed in equilibrated BM1 medium and then placed in 10 µl micro drops of the same medium in injection dishes, covered with 3 ml of sterile equilibrated mineral oil.

The oocyte grading: The oocyte was assessed quickly for maturity (Quality) according to grading system using an inverted (Olympus 1 × 71) microscope with Hoffman optics, hot stage and automatic manipulators Narishige.

D-Oocyte injection procedure: We examined and evaluated individual sperm subjected to ICSI. The injection procedure was carried out in a sterilized slide using hold pipette and injection needle. The mature oocyte put in 10 µl drop of BM1 medium at 37°C in a 5% CO_2 in a 100% humidity environment equilibrated and covered by mineral oil, was added to 10 µl drop of the PVP solution with 4-5 µl drop of the centrifuged sperm suspension from both origin (some of the oocytes with ejaculated sperm and the others with the testicular sperm). Intra-cytoplasmic sperm injection was performed according to the protocol of Van Steirteghem [18].

The injection procedure was carried out on A× overt 135, equipped with Hoffman optics, 10×, 20× and 40× objectives with 10× eye pieces and nourishing micromanipulators. The oocyte was attached to holding pipette using slight negative pressure. The injection needle containing the sperm in PVP was brought into the focal plan and a single sperm was positioned just at the tip of the microinjection needle.

The next step was a slow, steady and consistent movement into the cytoplasm of the metaphase 2(MII) oocyte. The sperm then was deposited into the cytoplasm with approximately 1 to 3 µl medium. The injected oocyte then washed twice in BMI medium covered with sterile warm equilibrated mineral oil at 37°C in a 5% CO_2 in a 100% humidity environment then put it in culture dish until fertilization examined.

Assessment of fertilization and embryo's quality: Fertilization was assessed 15-18 h after microinjection. The injected oocytes were observed for any sign of damage and for the presence of pro-nuclei. Oocytes were classed as fertilized if two pro-nuclei (2PN) were present and the second polar body had been extruded. Abnormally fertilized oocytes (1PN or 3PN) were excluded. Normally fertilized oocytes were left in culture for a further 24 h then embryos were classified according to a simplified system based on morphological criteria [19].

U/S done after embryo transfer by 4-5 weeks searching for gestational intrauterine sac.

Results

There was no statistical significant difference between group I and group II as regard to age, weight, height, duration of infertility, parity, abortion, previous ICSI, E2 baseline and FSH level.

Non-significant differences were found between females in the 1st and 2nd groups with respect to ICSI cycle parameters including the day of HCG injection, gonadotropin dose, estradiol level on the day of HCG administration (Tables 1 and 2).

Characteristic	Vitamin D status, mean (± SD)*		p value
	Group I	Group II	
25-Hydroxy-vitamin D nmol/L	83.277 ± 7.099	61.32 ± 8.877	<0.001*
Age	27.170 ± 3.179	27.06 ± 3.998	0.867

Weight	74.000 ± 15.261	73.85 ± 13.964	0.955
Height	153.585 ± 5.260	154.554 ± 6.899	0.384
Duration of infertility	5.057 ± 3.066	4.253 ± 2.987	0.132

Table 1: Characteristics of 136 women undergoing ICSI, by vitamin D status.

Number of oocytes was significantly higher among females with 1st group (mean 10.623 ± SD5.712) than among females in the 2nd group (mean 8.928 ± SD 3.083) respectively with (P-value 0.027) as shown in Table 3.

Increased clinical pregnancy rate per ICSI cycle started among females with 1st group (58.49%) than among females in the 2nd group (44.85%) respectively with (P-value 0.011 as shown in Table 3.

Statistical significant difference was found between (25[OH] D) in 1st group and 2nd group as regard to clinical pregnancy respectively with (P<0.001) as shown in Table 3.

Characteristic		**Vitamin D status, mean (± SD)***		**p value**
		Group I	**Group II**	
Parity	Yes	7.55%	10.84%	0.679
	No	92.45%	89.16%	
Abortion	Yes	1.89%	12.05%	0.138
	No	98.11%	87.95%	
Previous ICSI	Yes	18.87%	13.25%	0.377
	No	81.13%	86.75%	

Table 2: Characteristics of 136 women undergoing ICSI, by vitamin D status.

Characteristic	**Vitamin D status, mean (± SD)***		**p value**
	Group I	**Group II**	
Basal FSH	5.968 ± 1.653	5.694 ± 1.845	0.38
No. of follicles	10.623 ± 5.712	8.928 ± 3.083	0.027*
Day of HCG injection	14.660 ± 1.990	Sep-21	<0.001*
Basal E2 level	36.874 ± 8.927	35.496 ± 10.459	0.43
E2 level on the day of HCG administration	2265.566 ± 932.571	2365.976 ± 1116.118	0.587
Clinical pregnancy	85.619 ± 7.524	61.767 ± 9.033	<0.001*

Table 3: Characteristics of 136 women undergoing ICSI, by vitamin D status.

Discussion

Serum level of 25-hydroxy-vitamin D may be an ICSI predictor among females undergoing infertility treatment. Women in this cohort in group I had significantly higher of clinical pregnancy rates following ICSI compared with females in group II.

Findings are significant clinically and hold therapeutic implications as about 61% of females in our study had lower levels. These findings are agreed with those previously reported in a North American cohort of reproductive-age females who included 173 patients in analysis. All of the included females subjected to oocyte retrieval, and 162 subjected to embryo transfer. The prevalence of vitamin D deficiency, insufficiency and sufficiency was 53.8% and 45.1%, respectively. In the analyses, we grouped together females with deficient and insufficient 25-hydroxy-vitamin D levels [8].

These findings were confirmed in other study of a group of infertile Canadian women by Garbedian who subsequently underwent oocyte retrieval. Females who were 25-hydroxy-vitamin D deficient were grouped together with those who were 25-hydroxy-vitamin D insufficient. Females with sufficient 25-hydroxy-vitamin D levels had significantly higher clinical pregnancy rates per ART cycle than females with deficient and insufficient levels (52.5% versus 34.7%, p<0.001) [20].

Also Polyzos only divided women into two divisions: vitamin D deficient (<50 nmol/L) and not vitamin D deficient (≥ 50 nmol/L). Overall, 46% of patients achieved clinical pregnancy. Clinical pregnancy rates were significantly lower in vitamin D deficient women compared to their non-deficient counterparts (41% versus 54%, p=0.015) [21].

Also Anifandis and colleagues confirmed these findings in their study of a group of infertile women who subsequently underwent oocyte retrieval. Women with sufficient vitamin D levels had significantly higher rates of clinical pregnancy per ART cycle than women with insufficient/deficient levels (38.1% versus 20.8%, p<0.05) [10].

Also Ozkan Were the first to report a positive correlation between vitamin D levels and IVF outcomes. In their cohort prospective study, females achieving clinical pregnancy demonstrated significantly higher FF 25-hydroxy-vitamin D levels compared to females who did not achieve clinical pregnancy (≥ 75 nmol/L ± 15.58 versus <75 nmol/L ± 10.53, p=0.013) [4].

Unlike the previously discussed studies, Aleyasin found no relationship between serum vitamin D concentration and fertilization, implantation, or pregnancy rates in their study of 82 Iranian women [11]. A study conducted by Firouzabadi supported these findings. This cohort prospective study of 180 infertile females demonstrated no significant association between serum or FF 25-hydroxy-vitamin D levels and rates of clinical pregnancy. Overall, the rate of clinical pregnancy was 33.48%. (p=0.094) [21].

In currant study, we measure the serum levels of 25-hydroxy-vitamin D and not measure the level of 25-hydroxy-vitamin D in FF, While, other studies have shown the levels of 25-hydroxy-vitamin D in these fluids are highly correlated, and serum levels of 25-hydroxy-vitamin D have greater clinical utility. Future studies have to focus on determining the mechanism by which of 25-hydroxy-vitamin D affects rates of clinical pregnancy, and they should include investigate of embryo quality, implantation and uterine receptivity.

Conclusion

In our study population, we found higher prevalence of vitamin D deficiency or insufficiency. Thus, it may be beneficial to investigate vitamin D levels as part of routine infertility assessment and before ART treatment.

In current findings suggest that females with high levels of 25-hydroxy-vitamin D are significantly than those with insufficient levels to achieve clinical pregnancy following ICSI.

Recommendations

In current findings suggest that females with high levels of 25-hydroxy-vitamin D are significantly than those with insufficient levels to achieve clinical pregnancy following ICSI. Supplementation of vitamin D may be an easy and cost-effective way to improving rates of pregnancy.

Future studies should focus on determining the mechanism by which vitamin D affects clinical pregnancy, and they should include measures of embryo quality, implantation and uterine receptivity. Studies should also be performed to investigate whether vitamin D supplementation can improve the pregnancy rates following ICSI.

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