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ORIGINAL ARTICLE

Impact of Vitamin D Supplementation on Semen Quality in Vitamin D-Deficient Infertile Males with Oligoasthenozoospermia

Leena Wadhwa[1] · **Srishti Priyadarshini**[1] · **Ashish Fauzdar**[1] · **Sanjana N. Wadhwa**[1] · **Sarika Arora**[2]

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Abstract

Introduction Male factor contributes to almost 50% cases of infertility. There is evidence of the effect of serum vitamin D on male fertility by helping in spermatozoa maturation and initiating acrosomal reaction. In India, vitamin D deficiency has been documented to be in the range of 50–90% among all the age groups.

Objective To study the levels of vitamin D and the impact of vitamin D supplementation in vitamin D-insufficient/deficient infertile males with oligoasthenozoospermia.

Material and methods This study is a longitudinal observation study. Infertile men with either oligozoospermia or asthenozoospermia with vitamin D levels $<$ 30 ng/ml were enrolled. Vitamin D and calcium supplementation was given, and semen and hormone parameters were assessed at the end of 3 and 6 months.

Result There is significant improvement in the mean sperm concentration and progressive sperm motility in infertile males with oligoasthenozoospermia after 6 months of vitamin D supplementation (p value < 0.001). The overall clinical pregnancy rate in the study was 8.33% after vitamin D supplementation (p value 0.24).

Conclusion Vitamin D supplementation plays a crucial role in regulating male fertility.

Keywords Oligoasthenozoospermia · Sperm concentration · Sperm motility · Vitamin D deficiency · Male infertility

Introduction

As per WHO, all over the world approximately 60–80 million couples suffer from infertility [1]. Male factor contributes to almost 50% cases of infertility [2]. Multiple factors could contribute to the idiopathic cases of male infertility. Studies conducted have shown the presence of vitamin D receptors in the testis, prostate and mature spermatozoa (sperm nucleus and neck) [3]. The vitamin D metabolizing enzyme CYP24A1, which inactivates the active form of vitamin D, has been found at the annulus of the human spermatozoa [4].

In India, even though exposure to sunlight is ample, vitamin D deficiency has been documented to be in the range of 50–90% among all the age groups [5]. The main sources of vitamin D are sunlight, food and supplementation. The major causes of vitamin D deficiency in India include, dark-colored skin, poor sun exposure, food habits. Thus, supplementation of vitamin D remains a cornerstone in managing the deficiency of vitamin D. The objectives of the study were to study the levels of vitamin D and its impact on vitamin D supplementation in vitamin D-insufficient/deficient infertile males with oligoasthenozoospermia. This study also evaluated the effect of vitamin D supplementation on hormone and bone regulating parameters in same group of patients.

Dr. Leena Wadhwa, MD, DNB, MNAMS, FICOG is a Professor in Obstetrics and Gynaecology, ESIC-PGIMSR Basaidarapur, Delhi. Dr. Srishti Priyadarshini, MBBS is a Post graduate student in Obstetrics and Gyanecology, ESIC-PGIMSR Basaidarapur, Delhi. Dr. Ashish Fauzdar, Ph.D. is a Embryologist in Obstetrics and Gyanecology, ESIC-PGIMSR Basaidarapur, Delhi. Dr. Sanjana N. Wadhwa MBBS, DNB is a Assistant Professor in Obstetrics and Gyanecology, ESIC-PGIMSR Basaidarapur, Delhi. Dr. Sarika Arora, MBBS, MD Biochemistry is a Professor in ESIC-PGIMSR Basaidarapur, Delhi.

✉ Srishti Priyadarshini
doc.srishti187@gmail.com

[1] Department of Obstetrics and Gynaecology, ESIC-PGIMSR Basaidarapur, Delhi 110015, India

[2] ESIC-PGIMSR Basaidarapur, Delhi, India

Materials and Methods

The present study is a longitudinal observation study conducted at outpatient department, infertility clinic in the department of obstetrics and gynecology at tertiary care hospital. Men with idiopathic male factor infertility, in the age group of 23–40 years, with either oligozoospermia (sperm concentration < 15 million/ml) or asthenozoospermia (progressive sperm motility < 32%) or both were screened for levels of vitamin D. Men with vitamin D levels < 30 ng/ml were enrolled for the study. A total of 60 participants with male factor infertility and serum vitamin D levels < 30 ng/ml were enrolled for the study. A baseline level of serum vitamin D, semen parameters and hormone parameters were recorded. The cases were started on weekly 60,000 IU cholecalciferol and 500 mg daily calcium supplementation. After 3 months, vitamin D, semen and hormone parameters were reassessed. As none of the cases had vitamin D level > 30 ng/ml, the same therapy was continued for another 3 months. At the end of 6 months, the vitamin D levels, semen and hormonal parameters were measured and assessed.

Results

At the start of the study, 60 infertile men with oligoasthenozoospermia were included in the study; 25 out of them had serum vitamin D level < 20 ng/ml (vitamin D deficiency), and 35 had vitamin D levels between 20 and 29 ng/ml (vitamin D insufficiency). Infertile men with serum vitamin D levels > 30 ng/ml (vitamin D sufficiency) were not included in the study. After vitamin D supplementation for 3 months, the number of cases with vitamin D deficiency reduced to six cases from 25 and the other 19 became vitamin D insufficient. However, none of the cases were in vitamin D-sufficient range of serum vitamin D levels. After vitamin D supplementation for 6 months, none of the cases were in the vitamin D-deficient range, while 18 cases were still in vitamin D-insufficient range and 42 cases were in the vitamin D-sufficient range. The mean age of the study population was 30.57 years and mean BMI 22.95 kg/m^2. There was no statistically significant difference between the vitamin D-deficient and -insufficient males in terms of age, BMI, type of infertility and type of occupation ($p > 0.05$) (Table 1).

There was significant difference in the levels of serum vitamin D, progressive sperm motility, sperm concentration, serum phosphorus and serum alkaline phosphatase at the start of the study and after 3 and 6 months of vitamin D supplementation ($p < 0.05$) in both vitamin D-deficient and -insufficient groups. However, there was no statistically significant difference in the levels of serum calcium and reproductive hormones after 3 and 6 months of vitamin D supplementation in both the groups ($p > 0.05$) (Tables 2, 3, 4).

Correlation coefficient *r*: Analysis showed that there was a negative correlation between serum vitamin D levels and age over a period of 6 months with vitamin D supplementation. The correlation analysis showed a positive correlation between serum vitamin D levels and sperm concentration at the start of the study (r: 0.097; p: 0.463), after 3 months (r: 0.006; p: 0.962) and 6 months (r: 0.221; p: 0.09) of vitamin D supplementation. There was a positive correlation between serum vitamin D levels and progressive sperm motility at the start of the study (r: 0.116; p: 0.375), after 3 months (r: 0.109; p: 0.406) and 6 months (r: 0.144; p: 0.274) of vitamin D supplementation. There was a significant positive correlation between serum vitamin D levels and serum calcium level over a period of 6 months with vitamin D supplementation. There was a significant negative correlation between serum vitamin D levels and serum alkaline phosphatase over a period of 6 months with vitamin D supplementation; there was no significant correlation between serum vitamin D levels and reproductive hormone profile (Table 5).

Discussion

In various studies conducted, in both healthy and infertile male populations the mean serum vitamin D level was in the insufficient range of 61.25 nmol/l (Ramlau Hansen), 53 nmol/l (Blomberg Jensen), 21 ng/ml (Özdemir), 57.5 nmol/l (Blomberg Jensen) [6–9]. This is in contrast to

Table 1 Comparison of clinical characters between vitamin D-deficient and -insufficient infertile males with oligoasthenozoospermia

	Overall ($n = 60$)	Vitamin D deficient ($n = 25$)	Vitamin D insufficient ($n = 35$)	p value
Age (years)	30.57 ± 3.96	31.60 ± 3.62	29.83 ± 4.07	0.087
BMI (kg/m^2)	22.95 ± 2.44	23.26 ± 2.29	22.66 ± 2.54	0.280
Primary infertility	43	17	26	0.594
Secondary infertility	17	8	9	
Indoor activity	40	18	22	0.459
Outdoor activity	20	7	13	

Table 2 Effect of vitamin D supplementation on laboratory parameters in infertile males with oligoasthenozoospermia

$N=60$	Baseline	3 months	6 months	p value
S. vitamin D (ng/ml)	19.08 ± 4.98	29.42 ± 3.39	33.99 ± 6.23	0.003
Progressive sperm motility (%)	22.91 ± 1.88	30.57 ± 1.62	35.12 ± 1.40	< 0.001
Sperm concentration (million/ml)	19.72 ± 2.07	28.95 ± 2.06	33.63 ± 2.73	< 0.001
S. calcium (mg/dl)	8.99 ± 0.52	9.60 ± 0.44	11.62 ± 183	0.096
S. phosphorus (mg/dl)	3.23 ± 0.42	3.67 ± 0.45	4.22 ± 0.53	< 0.001
S. alkaline phosphatase (IU/L)	272.37 ± 59.51	243.52 ± 45.91	234.35 ± 40.28	< 0.001
LH (U/L)	7.71 ± 2.20	7.14 ± 1.25	7.17 ± 1.18	0.140
FSH (U/L)	7.46 ± 2.16	7.47 ± 1.70	7.48 ± 1.27	0.958
Testosterone (U/L)	2.53 ± 0.84	2.48 ± 0.71	2.59 ± 0.58	0.311
Estradiol (U/L)	28.90 ± 7.22	28.65 ± 5.26	29.36 ± 5.38	0.571

Table 3 Effect of vitamin D supplementation on laboratory parameters in vitamin D-deficient infertile males with oligoasthenozoospermia

$N=25$	Baseline	3 months	6 months	p value
S. vitamin D (ng/ml)	14.64 ± 4.61	21.80 ± 3.51	30.74 ± 5.33	0.03
Progressive sperm motility (%)	20 ± 1.6	32 ± 1.6	39 ± 1.2	< 0.001
Sperm concentration (million/ml)	19.72 ± 2.4	29.40 ± 2.08	36.20 ± 2.4	0.04
S. calcium (mg/dl)	8.74 ± 0.59	9.40 ± 0.39	9.87 ± 0.66	0.155
S. phosphorus (mg/dl)	3.34 ± 0.38	3.78 ± 0.41	4.30 ± 0.43	0.04
S. alkaline phosphatase (IU/L)	308.68 ± 57.84	264.76 ± 41.98	250.2 ± 38.29	0.002
LH (U/L)	8.27 ± 2.69	7.36 ± 1.29	7.22 ± 1.34	0.249
FSH (U/L)	7.62 ± 3.06	3.52 ± 2.21	7.34 ± 1.53	0.844
Testosterone (U/L)	2.62 ± 1.06	2.54 ± 0.74	2.54 ± 0.6	0.576
Estradiol (U/L)	29.44 ± 8.44	29.2 ± 6.04	29.19 ± 5.24	0.464

Table 4 Effect of vitamin D supplementation on laboratory parameters in vitamin D-insufficient infertile males with oligoasthenozoospermia

$N=35$	Baseline	3 months	6 months	p value
S. vitamin D (ng/ml)	22.25 ± 1.78	34.87 ± 4.37	36.31 ± 5.82	0.04
Progressive sperm motility (%)	25 ± 1.8	29 ± 1.7	32 ± 1.6	0.08
Sperm concentration (million/ml)	19.73 ± 1.88	28.63 ± 2.48	31.80 ± 2.37	0.04
S. calcium (mg/dl)	9.17 ± 0.37	9.74 ± 0.42	10.31 ± 0.44	0.446
S. phosphorus (mg/dl)	3.16 ± 0.35	3.59 ± 0.42	4.16 ± 0.48	0.03
S. alkaline phosphatase (IU/L)	246.43 ± 46.05	228.34 ± 42.95	223.03 ± 38.26	0.009
LH (U/L)	7.31 ± 1.7	6.98 ± 1.21	7.12 ± 1.08	0.747
FSH (U/L)	7.35 ± 1.21	7.43 ± 1.24	7.58 ± 1.07	0.477
Testosterone (U/L)	2.47 ± 0.64	2.43 ± 0.7	2.63 ± 0.58	0.585
Estradiol (U/L)	28.52 ± 6.3	28.25 ± 4.67	29.49 ± 5.55	0.837

the findings of the present study, wherein, the mean serum vitamin D level at the start of the study was 19.08 ng/ml, which was in vitamin D-deficient range. Factors such as lower socioeconomic status, pigmentation of skin can be the cause of lower mean serum vitamin D levels in the present study.

In the present study, the mean serum vitamin D levels were 19.08 ± 4.98 ng/ml at the start of the study at a mean age of 30.57 ± 3.96 years. Correlation analysis showed that there was a negative correlation between serum vitamin D levels and age (r: − 0.215; p: 0.1) similar to the findings of Özdemir et al. [8] (r: − 0.137; p: 0.054), thus indicating lower serum vitamin D levels with increasing age. In same study, among infertile men, a negative correlation was observed between vitamin D levels and BMI (r: −0.172, p: 0.01). However, no significant correlation was found between serum vitamin D levels and BMI in the present study (r: − 0.154; p: 0.241).

The studies undertaken to find the association between vitamin D levels and semen parameters show either a linear association (low semen parameters with low serum vitamin D levels and vice versa) or an inverse U association (low

Table 5 Correlation coefficient between clinical and laboratory parameters and level of serum vitamin D

	Baseline		3 months		6 months	
	r	*p*	*r*	*p*	*r*	*p*
Age	−0.215	0.1	−0.181	0.166	−0.084	0.524
BMI	−0.154	0.241	−0.073	0.581	0.021	0.871
Primary infertility	−0.101	0.44	−0.001	0.994	−0.009	0.948
Secondary infertility	−0.254	0.35	−0.078	0.774	−0.014	0.567
Indoor activities	−0.04	0.64	−0.08	0.247	−0.147	0.147
Outdoor activities	−0.104	0.638	−0.245	0.211	−0.146	0.367
Sperm concentration	0.097	0.463	0.006	0.962	0.221	0.09
Progressive sperm motility	0.116	0.375	0.109	0.406	0.144	0.274
S. calcium	0.428	0.001	0.483	<0.001	0.362	0.004
S. phosphorus	−0.212	0.104	−0.195	0.135	−0.285	0.027
S. alkaline phosphatase	−0.606	<0.001	−0.521	<0.001	−0.358	0.005
LH	−0.078	0.556	−0.117	0.375	0.06	0.648
FSH	−0.999	0.451	−0.048	0.717	−0.057	0.667
Testosterone	−0.015	0.912	−0.025	0.849	0.003	0.979
Estradiol	−0.027	0.838	−0.125	0.343	−0.4	0.761

semen parameters at both low and high serum vitamin D concentrations) In the study by Ramlau-Hansen et al. [6], among healthy men, there was an inverse U association between serum vitamin D levels and sperm concentration and progressive sperm motility. In the study by Hammoud et al. [10], in general population, inverse U association was found between serum vitamin D levels and sperm concentration and progressive sperm motility. Jensen et al. [9] showed an inverse U association in infertile males.

In the study by Özdemir et al. [8] among infertile males, linear association between serum vitamin D and semen parameters in infertile males was reported. In the present study, a linear association was observed between serum vitamin D and semen parameters. At baseline low mean serum vitamin D level (19.08 mg/dl) was associated with low mean progressive sperm motility (22.91%). However, the mean sperm concentration at baseline was 19.72 million/ml, which is above the lower reference limit. In the present study, the number of participants with oligoasthenozoospermia was 17 as compared to participants with asthenozoospermia, 23; thus, the mean levels of sperm concentration were above the lower reference limit. A negative correlation between serum vitamin D levels and sperm concentration was reported (*r*: −0.06; *p*: 0.392) in the study by Özdemir et al. [8]. In contrast to the study by Özdemir et al. [8], our study found a positive correlation between serum vitamin D levels and progressive sperm motility at the start of the study (*r*: 0.116; *p*: 0.375), after 3 months (*r*: 0.109; *p*: 0.406) and 6 months (*r*: 0.144; *p*: 0.274) of vitamin D supplementation and a positive correlation between serum vitamin D levels and sperm concentration at the start of the study (*r*: 0.097; *p*: 0.463), after 3 months (*r*: 0.006; *p*: 0.962) and 6 months (*r*: 0.221; *p*: 0.09) of vitamin D supplementation. While the study by Özdemir et al. was cross sectional, the present study supplemented infertile males with oligoasthenozoospermia with vitamin D for 6 months; hence, a positive correlation between vitamin D and semen parameters was seen in the present study.

The study by Deng et al. [11] showed a significant increase in sperm concentration and progressive sperm motility after 3 months of vitamin D supplementation in infertile males. In the study by Jensen et al. [12], there was no significant improvement in semen parameters after vitamin D supplementation. In the present study after supplementation of vitamin D for 3 months, the mean sperm concentration increased from 19.72 to 28.95 million/ml and after 6 months, increased to 33.63 million/ml ($p < 0.001$) and mean progressive motility increased from 22.91 to 30.57% while it increased to 35.15% after 6 months supplementation ($p < 0.001$).

In the study by Lerchbaum et al. [13], among healthy men, an inverse U association was observed between vitamin D and total testosterone levels. In the European Male Ageing Study [14], it was found that total testosterone levels increased linearly with increasing serum vitamin D levels and estradiol levels decreased linearly with increasing serum vitamin D levels. The study by Jensen et al. [9] did not find any significant association between the levels of serum vitamin D and total testosterone.

In the present study, there was no significant association between serum levels of vitamin D and levels of reproductive hormones. There was no significant correlation between serum vitamin D levels and reproductive hormones.

The effect of vitamin D supplementation on serum testosterone levels was studied by Pilz et al. [15]; after supplementation of vitamin D for 1 year, there was a significant improvement in the level of serum testosterone, from 10.7 ± 3.9 to 13.4 ± 4.7 nmol/l.

In contrast to the finding of the above study, the present study did not find any significant improvement in the levels of serum LH, FSH and testosterone and estradiol after supplementation of vitamin D for 6 months. The study by Pilz et al. [15] gave supplementation of vitamin D for 1 year as compared to the present study where supplementation was given for 6 months. This could explain the difference in effect of vitamin D supplementation on testosterone levels in the two studies.

In the present study, the overall clinical pregnancy rate in the study was 8.33% after 6 months of vitamin D supplementation; however, this was not statistically significant, *p*: 0.24. The clinical pregnancies in vitamin D-insufficient group were 5 out of 35 cases and in vitamin D-deficient group were 1 out of 25 cases, but this difference was not statistically significant. Despite improvement in semen parameters in the present study, no significant effect was in seen in clinical pregnancy rate as female factor infertility was not included in the study.

Limitations of the Study

The major limitation of the present study is a small sample size and the absence of a control group. The present study included only infertile males with impaired semen parameters with vitamin D deficiency, and there was no comparison of the effect of vitamin D supplementation on semen parameters in infertile males with fertile males. As this study was not a randomized trial, the results of the study cannot be extrapolated to all infertile males with impaired semen parameters. Further well designed and large series randomized control trials are needed to consider vitamin D as a novel therapeutic option for impairment of semen parameters.

Conclusion

The study included 60 infertile males with oligoasthenozoospermia after excluding other causes of male infertility. There was significant improvement in the mean sperm concentration and progressive sperm motility in infertile males with oligoasthenozoospermia after 6 months of vitamin D supplementation ($p < 0.001$). Despite improvement in semen parameters, there was no significant improvement in the levels of reproductive hormones and bone regulating parameters. The overall clinical pregnancy rate in the study was 8.33% after vitamin D supplementation, *p* value 0.24. Due to the inadequacy of appropriate controls and randomization, vitamin D supplementation cannot be considered for treatment of impaired semen parameters, yet. However, this study provides for further scope of research and trials in the role of vitamin D in improving semen parameters.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Standard All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Human and Animal Studies This article does not contain any study with animal subjects.

Informed Consent Informed consent was obtained from all patients for being included in the study.

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About the Author

Dr. Leena Wadhwa MD, DNB, MNAMS, FICOG, Professor, Obst & Gynae, ESI-PGIMSR, Basaidarapur New Delhi. Fellowships: FOGSI ETHICON FELLOWSHIP IN THE FIELD OF ENDOSCOPY. FOGSI-Dr VASANTBEN SHAH OVERSEAS SCHOLARSHIP Reproductive Medicine & Endoscopy-Bonn & Kiel Univ. Germany. Training in embryology, Chennai, India. Dr. Kamini Rao FOGSI Oration on Maternal Mortality at AICOG 2012. Book edited 'Current Concepts in Contraception & Women's Health'. Forty paper publications in International & National journals. RCT on endometrial scratching in IUI cycles included in Cochrane review 2016. Field of interest: Infertility, High-risk pregnancy and fertility enhancing endoscopic surgery. Site administrator & Web Editor of Indian Fertility Society. Executive member: Endometriosis & Infertility committee AOGD.