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Original Research Article

Comparison of vitamin D (25OHD) status between fertile and infertile men

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ABSTRACT

Background: Vitamin D (25OHD) deficiency has become a modern-day epidemic, being the most common nutritional deficiency worldwide. Many infertile men are experiencing low total sperm count or different semen abnormalities. The aim of this study was to compare serum vitamin D (25OHD) status among fertile and infertile men.

Methods: This was an observational (cross sectional comparative) study and was conducted in the Department of Reproductive Endocrinology and Infertility, BSMMU, Dhaka, Bangladesh during the period from April 2019 to March 2020. The sample size was 112 men where 56 participants were in fertile men group and 56 participants were infertile men group. Statistical analyses were carried out by using Windows based Statistical Package for Social Sciences (SPSS, version 23.0).

Results: The predictability of vitamin D insufficiency was significant. Holding the effects of vitamin D deficiency constant, males with vitamin D insufficiency were 3.28 times more likely to be infertile than males with vitamin D sufficiency. Subgroup analysis of infertile men was done regarding semen parameters in different vitamin D status categories. There was statistically significant difference in semen volume and sperm concentration between infertile men of different vitamin D status but no significant difference in case of motility and morphology.

Conclusions: There was no significant different of serum vitamin D (25OHD) between fertile and infertile men. Men with vitamin D insufficiency (≥ 20 ng/ml to < 30 ng/ml) are more likely to be infertile than men with vitamin D sufficiency.

Keywords: Fertile men and infertile men, Serum vitamin D (25OHD) status

INTRODUCTION

Infertility is defined as the failure to conceive after one year of unprotected intercourse with the same partner.¹ Male factor is one of the most frequent causes of infertility (40-50%). Many infertile men are experiencing low total sperm count or different semen abnormalities such as low sperm motility and impaired sperm function, thus resulting in inability to fertilize.² Vitamin D

(25OHD) is a fat-soluble steroid hormone involved in many functions of the body including calcium and phosphorous homeostasis, bone mineralization, cellular growth, and decreasing the risk for chronic illnesses such as diabetes, cardiovascular disease, cancer, obesity, and autoimmune diseases.³ The role of vitamin D in reproduction is an active area of investigation. Animal studies suggested that vitamin D (25OHD) deficiency in male rats may affect spermatogenesis and

supplementation improved testicular function.⁴ The presence of a vitamin D receptor (VDR) in several parts of the male reproductive system is likely to be related to various functions of the human reproductive axis.⁵ VDR and metabolizing enzymes are expressed in human spermatozoa.⁶ Interestingly, the expression of VDR and metabolizing enzymes is higher in spermatozoa from fertile than infertile men.⁷ On the other hand, evidence indicates that vitamin D deficiency may alter reproductive function indirectly through a calcium-dependent mechanism.⁸ The widespread vitamin D deficiency across all age groups and the published studies about the negative impact on overall health led to several investigations to assess the effect of vitamin D levels in human reproduction.⁹ Vitamin D deficiency has been advocated as a possible cause of male infertility in many studies conducted in the past several years. Nevertheless, a general consensus about the role of vitamin D in male infertility is still debated. Epidemiological studies reported an association between low or high vitamin D levels and semen parameters. Recent studies reported a relationship between vitamin D and poor semen quality in infertile men.^{10,11} In addition, vitamin D (25OHD) levels have shown a positive correlation with serum androgen levels in men. These observations have led to the hypothesis that vitamin D levels may be associated with sperm parameters.¹² The complete characterization of the association of vitamin D status with the male reproductive function remains to be elucidated. The purpose of this study is to compare serum vitamin D (25OHD) status among fertile and infertile men.

Objectives

General objective: To compare serum vitamin D (25OHD) status between fertile and infertile men.

Specific Objectives: To compare the serum vitamin D (25OHD) levels between fertile and infertile men. To categorize fertile and infertile males into three different vitamin D status (deficiency, insufficiency and sufficiency) according to vitamin D levels.

METHODS

This was an observational (cross sectional comparative) study and was conducted in the Department of Reproductive Endocrinology and Infertility, BSMMU, Dhaka, Bangladesh during the period from April 2019 to March 2020. The sample size was 112 men which was divided in two groups. 56 participants were in fertile men group and 56 participants were infertile men group. Statistical analyses were carried out by using Windows based Statistical Package for Social Sciences (SPSS, version 23.0).

Inclusion criteria for fertile men

Men whose youngest child not older than 1 year. Aged 20 to 40 years. Men who gave consent to participate.

Inclusion criteria for infertile men

Men with history of infertility and showing abnormal semen parameters at semen analysis. Aged 20 to 40 years. Men who gave consent to participate.

Exclusion criteria for fertile men

Any previous episode of involuntary infertility. Unwilling to participate voluntarily.

Exclusion criteria for infertile men

Men with age less than 20 and more than 40 years. Not willing to participate in the study. Men having history of mumps, trauma, surgery, un-descended testis, history of radiation, chemotherapy, drugs affecting sperm count and motility, sex and pituitary hormone use, brain surgery, pituitary surgery, renal disease and surgery, sexually transmitted infections.

The study was conducted by considering socio demographic variables and laboratory variables. Socio demographic variables were age, residence, education, occupation, income, duration of marriage, BMI, smoking status, and laboratory variables: semen parameters (semen volume, sperm count, motility and morphology), serum vitamin D (25OHD) levels, vitamin D status categories (deficiency, insufficiency and sufficiency). These are main outcome variables. A structured questionnaire was used in the study. It included all the variables of interests: a) Demographic data of the subjects i.e. age, BMI, duration of marriage, educationists; b) findings of physical examination: by a male doctor; c) findings of lab. Investigation: i.e. serum vitamin D (25OHD) level, semen parameter. After taking consent and matching eligibility criteria, data were collected from patients on variables of interest using the structured design by interview, observation, clinical examination and serum vitamin D (25OHD) level of the study groups. For infertile group necessary physical examination was done by a male doctor. Serum vitamin D (25OHD) level were measured of all study subjects using a commercially available it by immulite chemiluminescence immunoassay system in automated analyzer in National Institute of Nuclear Medicine and Allied Science, BSMMU.

Statistical analyses were carried out by using Windows based Statistical Package for Social Sciences (SPSS, version 23.0). Categorical variables described as frequency (percentage); continuous variables described as mean±SD, or median, range. Unpaired t test and ANOVA were done for continuous variables. Chi square test and Fishers exact test were done for categorical variables. Binary logistic regression analysis was done to see the predictability of different vitamin D status for fertility. A p value of less than 0.05 was considered significant. Confidentiality was maintained throughout. All data were kept anonymous.

RESULTS

In our study, there were 112 participants where 56 participants were in fertile and 56 participants were in

infertile group. Socio demographic characteristics of the two groups of fertile and infertile men are shown in Table 1.

Table 1: Socio demographic characteristics of study population (n=112).

Variables	Fertile men (n=56)	Infertile men (n=56)	P value			
Age (years)	33.54±4.46	32.63±4.16	^a 0.267 ^{ns}			
Duration of marriage (years)	5.14±2.62	5.86±3.52	^a 0.152 ^{ns}			
BMI (kg/m ²)	24.83±2.14	25.40±2.05	^a 0.226 ^{ns}			
Variables	Fertile men (n=56)		Infertile men (n=56)		P value	
	n	%	n	%		
Smoking status	Smoker	22	39.3	18	32.1	^b 0.430 ^{ns}
	Non-Smoker	34	60.7	38	67.9	
Education levels	Illiterate	1	1.8	0	0	^b 0.667 ^{ns}
	Primary	7	12.5	7	12.5	
	SSC	6	10.7	10	17.9	
	HSC	17	30.4	18	32.1	
	Above HSC	25	44.6	21	37.5	
Occupation status	Service	23	41.1	14	25	^b 0.449 ^{ns}
	Teacher	2	3.6	4	7.1	
	Business	4	7.1	6	10.7	
	No-occupation	1	1.8	1	1.8	
	Others	26	46.4	31	55.4	
Monthly income	10,000-20,000	26	46.4	18	32.1	^b 0.072 ^{ns}
	20,000-50,000	18	32.1	30	53.6	
	>50,000	12	21.4	8	14.3	
Location of residence	Urban	51	91.1	49	87.5	^b 0.541 ^{ns}
	Rural	5	8.9	7	12.5	

ns=not significant, ap value reached from Unpaired t test, bp value reached from Chi-square test

Table 2: Distribution of the study patients by serum vitamin D (25OHD) levels (n=112).

Serum vitamin D (25OHD) levels	Fertile man (n=56)	Infertile man (n=56)	P value
Mean±SD	20.03±9.14	22.57±8.35	0.12 ^{ns}
Median	18.38	21.8	
Minimum	2.02	3.5	
Maximum	38.05	51.66	
Skewness	0.329	0.779	
Kurtosis	-0.87	1.606	

ns=not significant

There were no statistical differences between the socio demographic variables of fertile and infertile men. We found that the mean serum vitamin D level was 20.03±9.14 ng/ml in fertile men and 22.57±8.35 ng/ml in infertile men. The difference was not statistically significant (p>0.05) between fertile and infertile group (Table 2). A two-way contingency table analysis (Table 3) was conducted to evaluate whether fertility of males was associated with vitamin D status. Two variables were vitamin D status with three levels (deficiency, insufficiency and sufficiency) and fertility with two

levels (fertile and infertile). A chi-square test of independence conducted between vitamin D status and fertility of males showed a statistically significant association between vitamin D status and fertility: χ^2 (2, n=192) =14.749 p=0.001. Phi and Cramer's V =0.277 (effect size small to moderate).

Table 3: Association of vitamin D status with fertility in males by 2×3 contingency analysis (Chi Square and Fishers Exact test).

Serum vitamin D (25OHD) levels	Fertile man (n=56)		Infertile man (n=56)		P value
	n	%	n	%	
<20 ng/mL vitamin D deficient	32	57.1	22	39.3	0.018
≥20 to <30 ng/ml vitamin D insufficient	11	19.6	25	44.9	
≥30 ng/ml vitamin D sufficient	13	23.2	9	16.1	

s=significant p value reached from Chi-square test

The association was significant, apparent with the status of vitamin D insufficient (69.4% in infertile men

compared to 30.6% in fertile men), on post-hoc analysis (Figure 1).

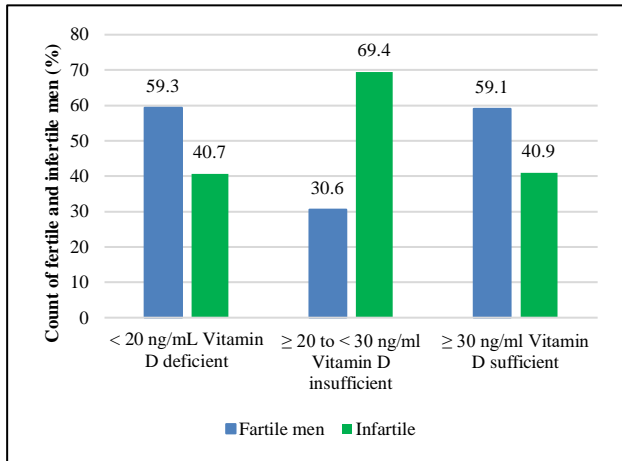


Figure 1: The relative frequency of fertile and infertile men in three categories of vitamin D status.

Table 4: The predictability of vitamin D deficiency and insufficiency compared to vitamin D sufficiency in males on the likelihood of being infertile (logistic regression analysis).

Variables	Significance of predictability	Odds ratios	95% CI for odds ratios
Vitamin D deficiency	0.989	0.993	0.362-2.722
Vitamin D insufficiency	0.001	3.283	1.085-9.930

Likewise, Fishers Exact test also showed a statistically significant relation between vitamin D status and fertility (p=0.001). Chi square test of independence has the violation of assumption where all cells should have expected counts greater than or equal to five which means there was not an adequate sample size to run the chi square test of independence which can maximize the risk of making a wrong decision. Vitamin D insufficiency was relatively more frequent in infertile men than in fertile men. However, this was not so for vitamin D deficiency, which was found to be more in fertile men. A binary logistic regression was performed to ascertain the predictability of vitamin D deficiency (serum vitamin D <20 ng/dl) and vitamin D insufficiency (serum vitamin D ≥20 ng/dl to <30 ng/dl) compared to vitamin D sufficiency (≥30 ng/dl) on the likelihood that the participants would be infertile. The predictability of vitamin D insufficiency was significant. The logistic regression model was statistically significant (Chi Square statistic 15.054 with degrees of freedom 2, p<0.001). The model explained 10.1% (Nagelkerke R square) of the variance in being infertile or fertile and correctly classified 63% of cases. Holding the effects of vitamin D deficiency constant, males with vitamin D insufficiency are 3.28 times more likely to be infertile than males with vitamin D sufficiency. The table listed the variables with significance of predictability, odds ratios and 95% confidence interval for odds ratios. Subgroup analysis (Table 5) of infertile men was done regarding semen parameters in different vitamin D status categories. There was statistically significant (p<0.05) difference in semen volume and sperm concentration between infertile men of different vitamin D status but no significant difference in case of motility and morphology.

Table 5: Comparison of semen parameters in infertile men (n=56) of different vitamin D status.

Semen parameters	Serum vitamin D (25OHD) status	n	%	Mean±SD	P value
Volume (ml)	<20 ng/ml	22	39.29	2.34±1.08	0.005 ^s
	≥20 to <30 ng/ml	25	44.64	2.41±1.00	
	≥30 ng/ml	9	16.07	3.74±1.29	
Concentration (M/ml)	<20 ng/ml	22	39.29	10.79±10.30	0.002 ^s
	≥20 to <30 ng/ml	25	44.64	19.32±25.85	
	≥30 ng/ml	9	16.07	40.76±22.96	
Motility (%)	<20 ng/ml	22	39.29	15.13±13.19	0.068 ^{ns}
	≥20 to <30 ng/ml	25	44.64	19.84±17.55	
	≥30 ng/ml	9	16.07	29.31±11.33	
Morphology (%)	<20 ng/ml	22	39.29	16.72±16.95	0.113 ^{ns}
	≥20 to <30 ng/ml	25	44.64	15.76±14.43	
	≥30 ng/ml	9	16.07	28.62±18.27	

s=significant, ns=not significant

DISCUSSION

There has been a growing interest in studying the association of vitamin D deficiency and infertility in recent years. It has been postulated that vitamin D

receptors (VDR) are found in human tissues such as male and female reproductive organs and play a major role in facilitating the biological activity of Vitamin D.^{3,13} Vitamin D deficiency has been advocated as a possible cause of infertility in many studies conducted in the past

several years. This cross-sectional comparative study was carried out with an aim to estimate serum vitamin D (25OHD) level in fertile men and infertile men, to find out any significant difference between the serum vitamin D (25OHD) level in fertile and infertile men and to compare vitamin D status in terms of deficiency, insufficiency and sufficiency in fertile and infertile man. The vitamin D levels in fertile and infertile men were 20.03±9.14 ng/ml and 22.57±8.35 ng/ml respectively. There was no significant difference between vitamin D levels in fertile and infertile men. Vitamin D insufficiency was significantly more in infertile men than in fertile men. Though the difference was insignificant, vitamin D deficiency was more frequent in fertile men than in infertile men. Based on these findings, the association of vitamin D status with male infertility could not be unequivocally established. The socio demographic characteristics were not statistically significant between fertile and infertile men in current study. So, the results of vitamin D were not influenced by it. Environmental factors could play a major role in the causes of male infertility.¹⁴ As sun exposure was sufficient around the year in most part of our country, seasonal variation may not affect vitamin D level in current study. There are studies from Bangladesh and Pakistan where urban people are found to have vitamin D levels much lower than that of rural people.^{15,16} The difference was not statistically significant between fertile and infertile men living in urban and rural areas. The present study findings were discussed and compared with previously published relevant studies. The mean vitamin D levels, both in fertile and infertile men were in insufficient range (≥ 20

ng/ml to >30 ng/ml). This may be due to predominance of urban men. There may be other factors like geographical placement of our country, duration and time of sun exposure that is influenced by sunbath, latitude, skin complexion, dietary habits, clothing styles, use of sun blocks etc. Regular sun exposure has decreased due to changing lifestyles. The study gives an insight into the prevalence of hypovitaminosis D in the heterogeneous population of fertile and infertile men in Bangladesh. The subclinical vitamin D deficiency is widely prevalent in India, Bangladesh and other countries of this part of the world. Studies reported mean serum vitamin D levels <20 ng/ml in 70-100% of apparently healthy subjects.¹⁷ The reasons may be the lifestyle practices such as clothing and habits that limit sun exposure. Dark skinned people have more melatonin, so less UV ray induced synthesis of vitamin D in skin. Vitamin D rich dietary sources like milk, animal fat is unaffordable or degraded by high phytate low calcium content or high heat dependent cooking practices. Vitamin D deficiency is pandemic, affecting both temperate and tropical countries. Almost half of the world's population has hypovitaminosis D.^{18,19} Like elsewhere, vitamin D deficiency is highly prevalent in south Asian countries.²⁰ According to a recently published review the prevalence of vitamin D deficiency in India ranges from 40% to 99%, with most of the studies reporting a prevalence of 80%-90%.²¹ A recent study conducted on Bangladeshi subjects reported that 86% participants had hypovitaminosis D; 61.4% had deficiency and 24.1% had insufficiency and Vitamin D level was found sufficient in 13.1% subjects, which is comparable to levels in fertile men of our study.²²

Table 6: Comparison of vitamin D levels in fertile and infertile men in various studies.

Author	Design and type	Study population	Age	Mean vitamin D (\pm SD)
Present study Bangladesh (2020)	Prospective observational	56 fertile, 56 infertile	33.54±4.46 (fertile) 32.63±4.16 (infertile)	20.03±9.14 (fertile) 22.57±8.35 (infertile)*NS
Shahraki et al²³ (2020) Iran	Prospective observational	112 fertile, 95 infertile	31±7.4(fertile) 30.1±5.1(infertile)	19.7±9.1(fertile) 17.4±8.8(infertile)**NS
Akhavizadegan et al (2017) Iran	Retrospective observational	116 fertile, 114 infertile	32±5(fertile) 34±6.037(infertile)	21±10 (fertile) 16±9 (infertile)*S
Mustafa et al (2017) Iraq	Prospective observational	17 fertile, 37 infertile	33±1.24 (infertile)	51.80±5.33 (fertile) 26.67±2.87 (infertile)*S
Yang et al¹⁰ (2012) China	Prospective observational	195 fertile, 364 infertile	30.5±3.5 (fertile) 30.3±3.3 (infertile)	54.13±14.27 (fertile) 53.24±14.53 (infertile)**NS

*S= Significant, **NS= Not Significant (difference in serum vitamin D levels of fertile and infertile men).

Current literature review showed only four studies which compared vitamin D status between fertile and infertile men. The findings are summarized in the following Table 6.

The difference of vitamin D levels in fertile and infertile men are not significant in our study, one reason for this may be small sample size. The present study categorized fertile and infertile males into three different vitamin D

status (deficiency, insufficiency and sufficiency) according to vitamin D levels and compared the categories of vitamin D status between fertile and infertile males. Only one study categorized fertile and infertile men into deficient (<20) and non-deficient (≥ 20) groups.²³ They found that vitamin D deficiency was significantly more in infertile than fertile men. The lack of significant difference in vitamin D deficiency between fertile and infertile men in our study can be attributed to

the small sample size (n=56 in each group). There were 112 fertile and 95 infertile men, a sample size much larger than ours. The strength of the study lies in the fact that binary logistic regression was done to see the predictability of different vitamin D status in determining fertility. Holding the effects of vitamin D deficiency constant, males with vitamin D insufficiency are 3.283 times more likely (95% confidence interval 1.085-9.93) to be infertile than males with vitamin D sufficiency. There are studies which explored the association of vitamin D levels with semen parameters in infertile men, some in fertile men as well. The findings are summarized for comparison with present study in Table 7. Three studies showed that serum vitamin D was not associated with semen parameters.²⁴⁻²⁶ The study population of one study was vitamin D sufficient young men (18 to 21 years

age). Another two studies had smaller sample size. All three studies were in European white population (Denmark, Italy, and Ireland respectively). It is not clear how the 25OHD influences the semen parameters. It may be by direct action or through the influence of reproductive hormones. The study proposed that the relationship between 25OHD and sperm motility and morphology might not be due to the direct action of 25OHD, but it exerts this action via ion-homeostasis.²⁷ A positive association is not always found between serum vitamin D levels and semen parameters. Vitamin D may have a positive effect on male fertility potential, through mechanisms such as sperm DNA integrity, estradiol levels or other possible factors not reflected in semen parameters.²⁸

Table 7: Association of different semen parameters with vitamin D: comparison of present study with other studies.

Author	Semen analysis	Concentration	Motility	Morphology	Volume
Present study (2020)	Infertile men	+	=	=	+
Shahraki et al ²³ (2020)	Fertile and Infertile men	=	=	=	=
Jueraitetibaik et al (2019)	Infertile men	+	=	=	=
Rehman et al (2018)	Infertile men	+	+	+	+
Jamali et al ¹⁶ (2018)	Infertile men	+	+	+	NA
Akhaviizadegan et al (2017)	Infertile men	+	+	+	+
Abbasihormozi et al (2017)	Infertile men	=	+	=	=
Mustafa et al (2017)	Fertile and Infertile men	+	+	+	NA
Tirabassi et al (2017)	Infertile men	NA	+	NA	NA
Blomberg et al ⁵ (2016)	Infertile men	+	+	=	=
Zhu et al (2016)	Infertile men	+	+	NA	NA
Hammoud et al ¹² (2012)	Infertile men	+	+	=	=
Yang et al ¹⁰ (2012)	Fertile and Infertile men	NA	+	+	=

+: significant positive association, =: no association, NA: not assessed.

In our study there was small sample size and absence of random sampling, so risk of selection bias. There was only a single center, so may not represent wider population. Semen analysis of fertile men was not done. This study was the first comparative study in our country setup, men having child were not willing and comfortable with giving semen

CONCLUSION

Serum vitamin D (25OHD) levels are not significantly different among fertile and infertile men. Semen volume and sperm concentration show significant difference in infertile men of different vitamin D status. Men with vitamin D insufficiency (≥ 20 ng/ml to >30 ng/ml) are more likely to be infertile than men with vitamin D sufficiency. Vitamin D supplementation may have a favourable effect on fertility. Cohort and case control studies will be more appropriate than cross sectional studies to define the causal association of vitamin D status with infertility. Randomized controlled trials of vitamin D supplementation in infertile men will add to clinical significance of the association. Large multicenter

well-designed studies with larger sample size can be undertaken.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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