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Vitamin D3: Association of Low Vitamin D3 Levels with Semen Abnormalities in Infertile Males

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Abstract

Background: Insufficiency of vitamin D is one of the utmost general health issues all over the globe, including Pakistan; incompatible data are present on the possible relationship among serum vitamin D values and quality of semen. **Objective:** Lack of Vitamin D in body is related with a higher risk of various health problems including infertility. In our setup no such type of study had been conducted in the past on the relationship among serum vitamin D levels and infertility. This study was aimed to investigate male subjects with infertility and serum levels of vitamin D. **Methods:** This study was cross-sectional and performed on 243 male subjects who attended the clinic for evaluation of infertility from January 2016 to December 2017. Mean age of patients was 31.94 years with standard deviation of 7.52 years. The mean age of controls was 32.2 years with SD 8.42 years, age ranged between 20 years as minimum to 46 years as maximum. The minimum vitamin D level was 7.00 ng/dl while maximum 130 ng/dl, mean was 26.20 and standard deviation was 22.06 ng/ml. Levels of vitamin D were significantly low in (67%) male subjects with infertility. The mean vitamin D level of controls was 49.40 with SD of 35.98 ng/ml. 28% of controls had also vitamin D levels below the normal range, which indicates the prevalence of vitamin D deficiency in general population here in our setup. The participants were divided according to WHO (World Health Organization) 2010 criteria along with corresponding healthy control subjects. A questionnaire for evaluation of infertility in male subjects was filled by all the contestants. **Results:** Levels of vitamin D were significantly low in (67%) male subjects with infertility. While 28% of controls had also vitamin D levels below the normal range. Infertile male subjects with low or no sperm count were found insufficient for vitamin D in comparison to healthy control subjects. **Conclusion:** It was concluded from the results of current study that low levels of vitamin D are related with abnormal male reproductive function, the severity of that was subject to the amount of vitamin D insufficiency.

Keywords

Vitamin D Deficiency, Sperm Motility, Male Infertility

1. Introduction

Insufficiency of vitamin D is one of the utmost general health issues all over the globe, including Pakistan. Incompatible data are present on the possible relationship among serum vitamin D values and quality of semen with sperm motility. Lack of vitamin D was common amongst male subjects with infertility. Additional vitamin D and calcium may be the suitable treatment for subjects with post washed out total progressively motile sperm count (TPMSC) less than 5 million/ml with documented Vitamin D and Calcium deficiency [1].

Insufficiency of vitamin D is well thought-out as the most important community health issue in both developed and under developing nations. In the tropical regions of the Persian Gulf as Iran, Bahrain and Saudi Arabia this is considered as widely spread health issue; because of changes in dietary habits, lifestyle alterations and also due to industrialization [2].

Daylight exposure, clothing, skin pigmentations, geographical latitude, seasons, daily nutritional intake of Vitamin D, air contamination as well as overweight are the different factors related with different levels of vitamin D between different populations [3] [4] [5].

In tropical regions the incidence of vitamin D deficiency (≤20 ng/ml) ranges between 30% and 90% [3] [6]. In human spermatozoa Vitamin D Receptor (VDR) and other metabolic enzymes are located [7] [8] [9]. There is a relationship among different parameters of semen with low or high levels of vitamin D [10] [11] [12]. The semen quality depends upon the levels of vitamin D in serum; lack of this vitamin can alter the reproductive system through calcium dependent mechanism [9]. Studies had been conducted all over the world regarding this issue but our setup is lacking it. In current study vitamin D3 levels will be assessed in infertile male subjects to assess the occurrence of low levels of vitamin D3 in these subjects.

1.1. Objective

The present research was aimed to evaluate the association of low levels of vitamin D3 with sperm count and sperm motility in Pakistani infertile males.

1.2. Operational Definitions

1) Male Infertility:

Failure to conceive after one year of unprotected intercourse with the same partner is defined as male infertility [13].

2) Vitamin D3 deficiency: [14]

Analysis of serum concentrations of 25(OH) D through laboratory.

Normal > 30 ng/ml,

Insufficiency > 20 and < 30 ng/ml,

Deficiency < 20 ng/ml.

2. Material and Methods

1) Design of study: The current research was cross sectional.

2) Place of study: Peoples Medical College Hospital Nawabshah.

3) Duration of study: January 2016 to December 2017.

4) Inclusion criteria: All young and middle aged males with history of infertility and semen detailed report, showing evidence of infertility in its different parameters. Having no co morbid in relation to vitamin D related like liver diseases endocrine disease metabolic disease etc. All the male patients who were reported for semen analysis due to infertility and having semen abnormality were included for vitamin D levels. Simultaneously control group was added of same age and gender for vitamin D analysis.

5) Exclusion criteria: Subjects with age less than 20 and more than 60 years, not willing to participate in the study. Subjects 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.

6) Data collection:

After ethical consideration and permission this study was performed at PMCH Nawabshah. This was cross-sectional type of study; 243 male subjects who presented to clinic for assessment of infertility were included in this study from January 2106 to December 2017.

Rao-software for sample size calculation was used to calculate sample number. Total population of district Shaheed Benazirabad is approximately 1,600,000, with confidence interval of 95%, 50% response distribution a sample size of 243 subjects with infertility were selected for study after fulfilling the inclusion criteria.

141 normal fertile subjects of same age and sex were selected as control group for to compare the level of vitamin D3 in control subjects and patients having no

co morbid state.

According to WHO (World Health Organization) 2010 criteria the contestants were with abnormal sperm count. Samples for semen analysis were taken after sexual abstinence of 2 - 5 days as established upon World Health Organization (WHO) criterion (2010) [15].

Data was collected on pre designed proforma; every contestant provided informed written consent before the beginning of study. Questionnaires for dietary habits were also completed by subjects.

Samples of blood and semen were collected for analysis.

Results for semen analyses and vitamin D3 were entered on a proforma simultaneously to check the relation of sperm count and quality with vitamin D levels.

Blood was collected from a vein and was propelled to laboratory for the assessment of vitamin D3. Mini Vidas Biomerieux Global Company France was used for serum vitamin D3 assessment. Hypovitaminosis D was labeled when serum vitamin D levels were <30 ng/ml.

The semen was collected and analyzed for gross and microscopic examination for different parameters of semen.

7) Data analyses:

All the collected data were assessed by SPSS (Statistical Package for Social Science) version 20.0.Variables (categorical) as infertile males and serum levels of vitamin D were computed for assessment of frequency and percentages. Clinical, qualitative and quantitative variables were calculated for Mean ± SD, median and percentages for sperm count.

Mean and standard deviation were aimed for quantitative variables like, age and levels of vitamin D3. The t-test, one-way Anova were used to compare in between groups. Pearson correlation analyses were done to evaluate the relationship among various parameters.

Importance of serum levels of vitamin D were perceived for age, infertility, semen analyses with different parameters to see the impact of these on outcomes. $P < 0.05$ was taken statistically significant. Chi-square test was used for the assessment of differences in ratios. Association amongst serum levels of vitamin D3, male infertility and semen analysis were explored through bivariate correlation analysis.

3. Results

3.1. Descriptive Statistics

In this current study 243 subjects presented with infertility, all were male, the minimum age was 20 years while maximum age was 45 years, mean age was 31.94 years with standard deviation of 7.52 years. The mean age of controls was 32.2 years with SD 8.42 years, age ranged between 20 years as minimum to 46 years as maximum.

The minimum marriage duration was 1 year while maximum 25 years, mean marriage duration was 31.94 years and standard deviation was 5.70. The minimum vitamin D level was 7.00 ng/dl while maximum 130 ng/dl, mean was 26.20 and standard deviation was 22.06 ng /ml.

The mean vitamin D level of controls was 49.40 with SD of 35.98 ng/ml. 28% of controls had also vitamin D levels below the normal range, which indicates the prevalence of vitamin D deficiency in general population here in our setup.

The minimum serum calcium level was 07.2 while maximum 10.80, mean was 08.82 and standard deviation was 0.96 mg%. The mean serum calcium of control was 09.4 with SD of 0.88 mg%.

The minimum semen quantity was 1ml while maximum 5ml, mean was 2.07 ml and standard deviation was 1.40 ml. The minimum total sperm count was 0.00 million while maximum was 120 millions, mean was 25.91 and standard deviation was 26.41. The minimum percentage of motile sperms was 0.00% while maximum was 80%, mean was 29.73 and standard deviation was 26.33. The minimum percentage of rapid linear progression of sperms was 0.00% while maximum 20.00% mean was 2.41 and standard deviation was 3.75. The minimum percentage of slow non linear progression was 0.00% while maximum was 60%, mean was 12.11 and standard deviation was 13.11. The minimum percentage of non motile non progressive sperms was 0.00% while maximum was 100%, mean was 61.60 and standard deviation was 36.83. The minimum percentage of dead sperms was 0.00% while maximum was 95%, mean was 48.00 and standard deviation was 33.70. The minimum percentage of normal forms of sperms was 0.00% while maximum 100% mean was 49.40 and standard deviation was 35.98. The minimum percentage of abnormal sperms was 0.00% while maximum was 95%, mean was 28.78 and standard deviation was 29.02. The minimum germ cell per high power field (HPF) was 0.00 while maximum was 2.0, mean was 0.32 and standard deviation was 0.65. The minimum RBCs per high power field (HPF) was 0.00 while maximum was 10.00, mean was 3.20 and standard deviation was 2.14. The minimum pus cells per high power field (HPF) was 2 while maximum was 10.0, mean was 4.78 and standard deviation was 2.07. The minimum average sun exposure duration/24 hours was 01 hour while maximum was 05 hours, mean was 1.71 and standard deviation was 1.08 (Table 1).

3.2. Demographic Statistics

In Table 2 it is observed that 187 (77%) males were belonging to young age group while 56 (23%) were belonging to middle age group. History of sun exposure was <01 hour in 147 (60.5%), 2 - 3 hours in 49 (20.2%), 3 - 4 hours in 26 (10.7%), 4 - 5 hours in 11 (4.5%) and >5 hours in 10 (4.1%) subjects. A total of 219 (90.1%) belonged to rural setup while 24 (9.9%) were urban. Socioeconomic class had shown dominant ratio of lower economic class 221 (90.9%), while 15(6.2%), 7 (2.9%) belonged middle and upper class respectively. Occupation wise percent had shown that 126 (51.9%) were indoor workers while 90 (37%)

Table 1. Descriptive statistics n = 243.

	Minimum	Maximum	Mean	Std. Deviation
Age In Years	20.00	45.00	31.9465	7.52470
Marriage Duration In Years	1.00	25.00	9.0947	5.70661
Vitamin D Level	7.00	130.00	26.2025	22.06323
Serum Calcium Level	7.20	10.80	8.8210	0.96446
Semen Quantity 2 - 4 Ml	1.00	5.00	2.0741	1.40346
Total Sperm Count (Normal 40 - 200 M/Ml)	0.00	120.00	25.9136	26.41462
Motile Sperm (Normal 70% - 90%)	0.00	80.00	29.7325	26.33287
Rapid Linear Progression (Normal > 25%)	0.00	20.00	2.4115	3.75095
Slow/Non Linear Progression	0.00	60.00	12.1111	13.11929
Non Progressive	0.00	100.00	61.6008	36.83593
Dead Sperm %	0.00	95.00	48.0041	33.70123
Normal Form (>4%)	0.00	100.00	49.4033	35.98602
Abnormal Sperm %	0.00	95.00	28.7860	29.04235
Germ Cells	0.00	2.00	0.3251	0.65340
RBCs (HPF)	0.00	10.00	3.2016	2.14853
Pus cells (HPF)	2.00	10.00	4.7819	2.07249
Month Of Year	1.00	12.00	5.2140	3.91905
Sun Exposure	1.00	5.00	1.7160	1.08979

Table 2. Frequency and percentage of demographic variables n = 243.

variable		Frequency	Percent
age group	20 - 40 years young age	187	77
	41 - 60 years middle age	56	23
	Total	243	100
sun exposure	<1 hr/day	147	60.5
	2 - 3 hr/day	49	20.2
	3 - 4 hr/day	26	10.7
	4 - 5 hrs/day	11	4.5
	>5 hrs/day	10	4.1
	Total	243	100
address	Rural	219	90.1
	Urban	24	9.9
	Total	243	100
socioeconomic class	lower class	221	90.9
	middle class	15	6.2
	upper class	7	2.9
	Total	243	100

Continued

occupation	no occupation	27	11.1
	indoor worker	126	51.9
	outdoor worker	90	37
	Total	243	100
vitamin D Deficiency	>more than 30 ng/dl normal	79	32.5
	20 - 30 ng/dl insufficient	17	7
	<20 ng/dl deficient	147	60.5
	Total	243	100

were out door workers and remaining 27 claimed no occupation. Vitamin D levels were within normal or sufficient range in 79 (32.5%), it was below normal range in 164 (67.5%) cases, while insufficient in 17 (7.0%) and deficient in 147 (60.5%) cases of infertility Table 2.

3.3. Vitamin D and Semen Quantity

Numbers of valid cases were 243 infertile males. The quantity of semen in ml were counted 126 cases have semen quantity 1 ml, 46 cases 2 ml, 29 cases 3 ml, 11 cases 4 ml and 31 cases 5 ml. Out of 126 cases having semen quantity 1 ml, 51 had normal range vitamin D level, while 75 cases were below normal range. From 46 cases having 2ml semen per ejaculate 9 cases has normal vitamin D levels, while 37 with lower than normal. From 29 cases having 3ml semen per ejaculate 6 cases has normal vitamin D levels, while 23 with lower than normal. From 11 cases having 4ml semen per ejaculate 4 cases has normal vitamin D levels, while 7 with lower than normal. From 31 cases having 5 ml semen per ejaculate 9 cases has normal vitamin D levels, while 22 with lower than normal. Vitamin D levels were normal in 79 cases of infertility while they were insufficient and deficient in 17 and 147 cases respectively. Pearson chi square was 10.026, df 8, Asymp. sig. (2-sided) 0.263. Likelyhood ratio 10.277 df 8, Asymp. sig. (2-sided) 0.246. Linear by linear association was 2.202, df 1, Asymp. sig. (2-sided) 0.138. Interval by interval pearsons R value was 0.095, Approx. Sig 0.138. Ordinal by ordinal Spearman correlation value was 0.130, Approx. Sig 0.042 (Table 3).

3.4. Vitamin D and Total Sperm Count

Out of 243 cases 53 (21.8%) were having sperm count nil. While 190 (79.2%) cases having different count of sperm ranging from 02 million to 120 million. From these subjects 79/243 had normal range vitamin D levels while 164 had below normal range vitamin D levels. Pearson chi square was 175.201, DF 28, Asymp. sig. (2-sided) $p < 0.001$. Likelyhood ratio 182.638, df 28, Asymp. sig. (2-sided) $p < 0.001$ Linear by linear association was 94.038, df 1, Asymp. sig. (2-sided) $p < 0.001$ Interval by interval pearsons R value was −0.623, Approx.

Table 3. Vitamin D deficiency and semen quantity (normal 2 - 4 ml) n = 243.

			semen quantity 2 - 4 ml					Total
			1.00	2.00	3.00	4.00	5.00	
vitamin D Deficiency	>more than 30 ng/dl normal	Count	51	9	6	4	9	79
		% within vitamin D Deficiency	64.6%	11.4%	7.6%	5.1%	11.4%	100.0%
	20 - 30 ng/dl insufficient	Count	7	5	2	1	2	17
		% within vitamin D Deficiency	41.2%	29.4%	11.8%	5.9%	11.8%	100.0%
	<20 ng/dl deficient	Count	68	32	21	6	20	147
		% within vitamin D Deficiency	46.3%	21.8%	14.3%	4.1%	13.6%	100.0%
Total		Count	126	46	29	11	31	243
		% within vitamin D Deficiency	51.9%	18.9%	11.9%	4.5%	12.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.026[a]	8	0.263
Likelihood Ratio	10.277	8	0.246
Linear-by-Linear Association	2.202	1	0.138
N of Valid Cases	243		

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	0.095	0.064	1.488	0.138[c]
Ordinal by Ordinal	Spearman Correlation	0.130	0.064	2.043	0.042[c]

Sig. p < 0.001 Ordinal by ordinal Spearman correlation value was −0.541, Approx. Sig p < 0.001 Table 4.

3.5. Vitamin D and Sperm Motility

Regarding the sperm motility normal range was 70% - 90%. Over all Pearson chi square was 103.788, df 22, Asymp. sig. (2-sided) p < 0.001. Out of 243 cases 53 (21.8%) were having sperm count nil. While 190 (79.2%) cases having different percentage of sperm motility ranging from 5% - 80%. From these subjects 79/243 had normal range vitamin D levels while 164 had below normal range vitamin D levels. Likelyhood ratio 117.412 df 22, Asymp. sig. (2-sided) p < 0.001. Linear by linear association was 44.488, df 1, Asymp. sig. (2-sided) p < 0.001. Interval by interval pearsons R value was −0.429, Approx. Sig p < 0.001. Ordinal by ordinal Spearman correlation value was −0.414, Approx. Sig p < 0.001 Table 5.

Table 4. Vitamin D deficiency and total sperm count (normal 40 - 200 M/Ml) n = 243.

			Total Sperm Count (normal 40 - 200 M/Ml)														Total
			0.00	2.00	6.00	12.00	15.00	34.00	40.00	42.00	45.00	50.00	60.00	70.00	80.00	90.00	120.00	
vitamin D Deficiency	>more than 30 ng/dl normal	Count	11	0	1	3	0	7	1	11	1	15	4	4	14	5	2	79
		% within vitamin D Deficiency	13.9%	0.0%	1.3%	3.8%	0.0%	8.9%	1.3%	13.9%	1.3%	19.0%	5.1%	5.1%	17.7%	6.3%	2.5%	100.0%
	20 - 30 ng/dl insufficient	Count	2	0	0	3	2	10	0	0	0	0	0	0	0	0	0	17
		% within vitamin D Deficiency	11.8%	0.0%	0.0%	17.6%	11.8%	58.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%
	<20 ng/dl deficient	Count	40	5	20	21	34	10	0	16	0	0	0	0	1	0	0	147
		% within vitamin D Deficiency	27.2%	3.4%	13.6%	14.3%	23.1%	6.8%	0.0%	10.9%	0.0%	0.0%	0.0%	0.0%	0.7%	0.0%	0.0%	100.0%
Total		Count	53	5	21	27	36	27	1	27	1	15	4	4	15	5	2	243
		% within vitamin D Deficiency	21.8%	2.1%	8.6%	11.1%	14.8%	11.1%	0.4%	11.1%	0.4%	6.2%	1.6%	1.6%	6.2%	2.1%	0.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	175.201[a]	28	0.000
Likelihood Ratio	182.638	28	0.000
Linear-by-Linear Association	94.038	1	0.000
N of Valid Cases	243		

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	−0.623	0.045	−12.376	0.000[c]
Ordinal by Ordinal	Spearman Correlation	−0.541	0.057	−9.982	0.000[c]

Table 5. Vitamin D deficiency and motile sperm (normal 70% - 90%) n = 243.

			motile sperm (normal 70% - 90%)												Total
			0.00	5.00	10.00	20.00	30.00	35.00	40.00	45.00	60.00	65.00	70.00	80.00	
vitamin D Deficiency	>more than 30 ng/dl normal	Count	11	1	0	1	20	1	2	1	17	1	20	4	79
		% within vitamin D Deficiency	13.9%	1.3%	0.0%	1.3%	25.3%	1.3%	2.5%	1.3%	21.5%	1.3%	25.3%	5.1%	100.0%
	20 - 30 ng/dl insufficient	Count	2	0	0	2	10	0	0	0	0	0	3	0	17
		% within vitamin D Deficiency	11.8%	0.0%	0.0%	11.8%	58.8%	0.0%	0.0%	0.0%	0.0%	0.0%	17.6%	0.0%	100.0%
	<20 ng/dl deficient	Count	40	25	4	30	26	0	0	0	0	0	22	0	147
		% within vitamin D Deficiency	27.2%	17.0%	2.7%	20.4%	17.7%	0.0%	0.0%	0.0%	0.0%	0.0%	15.0%	0.0%	100.0%
Total		Count	53	26	4	33	56	1	2	1	17	1	45	4	243
		% within vitamin D Deficiency	21.8%	10.7%	1.6%	13.6%	23.0%	0.4%	0.8%	0.4%	7.0%	0.4%	18.5%	1.6%	100.0%

Chi-Square Tests

Continued

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	103.788[a]	22	0.000
Likelihood Ratio	117.412	22	0.000
Linear-by-Linear Association	44.488	1	0.000

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	−0.429	0.059	−7.368	0.000[c]
Ordinal by Ordinal	Spearman Correlation	−0.414	0.059	−7.068	0.000[c]

3.6. Vitamin D and Normal Sperm Count

The normal forms were considered if they are more than 4%. In 21.8% there were no sperm at all; while in rest there were different percentages of normal sperms ranging from 5% to 100%. Only 1.2% cases were with 100% normal forms , while others were with different percentages as shown in Table 6, 79.2% cases were of normal forms but out of them 79 were with normal range of vitamin D and rest of all 164/243 were vitamin D deficient. Pearson chi square was 96.799, df 20, Asymp. sig. (2-sided) $p < 0.001$ Likelihood ratio 112.519, df 20, Asymp. sig. (2-sided) $p < 0.001$, Linear by linear association was 37.007, df 1, Asymp. sig (2-sided) $p < 0.001$. Interval by interval pearsons R value was −0.391, Approx. $p < 0.001$. Ordinal by ordinal Spearman correlation value was −0.445, Approx. Sig $p < 0.001$ (Table 6).

3.7. Vitamin D and Abnormal Sperm Count

Abnormal sperm morphology had been observed in semen analysis, in context to vitamin D levels. 23.0% cases had no abnormality while 775 cases were with different percentages of abnormality ranged from 10% to 95%. Pearson chi square was 88.583, df 18, Asymp. sig. (2-sided) $p < 0.001$. Likelyhood ratio 103.671 df 18, Asymp. sig. (2-sided) $p < 0.001$ Linear by linear association was 17.195, df 1, Asymp. sig. (2-sided) $p < 0.001$ Interval by interval pearsons R value was .267, Approx. Sig $p < 0.001$. Ordinal by ordinal Spearman correlation value was .225, Approx. Sig $p < 0.001$ (Table 7).

3.8. Vitamin D and Dead Sperm Count

In current study there were 21.8% cases with no sperm, while 78.2 percent with different percentages of dead sperms ranged from 20% to 95% as shown in Table 8 in relation to different levels of vitamin D. Pearson chi square was 122.850, df 20, Asymp. sig. (2-sided) $p < 0.001$. Likelihood ratio 135.612 df 20, Asymp. sig. (2-sided) $p < 0.001$ Linear by linear association was 6.869, df 1, Asymp. sig. (2-sided) $p < 0.001$. Interval by interval pearsons R value was 0.168, Approx. Sig $p < 0.008$, Ordinal by ordinal Spearman correlation value was 0.203, Approx. Sig $p < 0.001$ (Table 8).

Table 6. Vitamin D deficiency and normal form sperms n = 243.

			Normal form (>4%)											Total
			0.00	5.00	10.00	15.00	55.00	70.00	75.00	80.00	85.00	90.00	100.00	
vitamin D Deficiency	>more than 30 ng/dl normal	Count	11	1	1	1	0	1	19	25	5	12	3	79
		% within vitamin D Deficiency	13.9%	1.3%	1.3%	1.3%	0.0%	1.3%	24.1%	31.6%	6.3%	15.2%	3.8%	100.0%
	20 - 30 ng/dl insufficient	Count	2	0	0	0	2	0	3	10	0	0	0	17
		% within vitamin D Deficiency	11.8%	0.0%	0.0%	0.0%	11.8%	0.0%	17.6%	58.8%	0.0%	0.0%	0.0%	100.0%
	<20 ng/dl deficient	Count	40	29	0	0	30	0	21	26	0	1	0	147
		% within vitamin D Deficiency	27.2%	19.7%	0.0%	0.0%	20.4%	0.0%	14.3%	17.7%	0.0%	0.7%	0.0%	100.0%
Total		Count	53	30	1	1	32	1	43	61	5	13	3	243
		% within vitamin D Deficiency	21.8%	12.3%	0.4%	0.4%	13.2%	0.4%	17.7%	25.1%	2.1%	5.3%	1.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	96.799[a]	20	0.000
Likelihood Ratio	112.519	20	0.000
Linear-by-Linear Association	37.007	1	0.000

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	−0.391	0.056	−6.596	0.000[c]
Ordinal by Ordinal	Spearman Correlation	−0.445	0.057	−7.707	0.000[c]

Table 7. Vitamin D deficiency and abnormal sperm% n = 243.

			abnormal sperm %										Total
			0.00	10.00	15.00	20.00	25.00	30.00	45.00	85.00	90.00	95.00	
vitamin D Deficiency	>more than 30 ng/dl normal	Count	14	12	5	25	19	1	0	1	1	1	79
		% within vitamin D Deficiency	17.7%	15.2%	6.3%	31.6%	24.1%	1.3%	0.0%	1.3%	1.3%	1.3%	100.0%
	20 - 30 ng/dl insufficient	Count	2	0	0	10	3	0	2	0	0	0	17
		% within vitamin D Deficiency	11.8%	0.0%	0.0%	58.8%	17.6%	0.0%	11.8%	0.0%	0.0%	0.0%	100.0%
	<20 ng/dl deficient	Count	40	1	0	26	21	0	30	0	0	29	147
		% within vitamin D Deficiency	27.2%	0.7%	0.0%	17.7%	14.3%	0.0%	20.4%	0.0%	0.0%	19.7%	100.0%
Total		Count	56	13	5	61	43	1	32	1	1	30	243
		% within vitamin D Deficiency	23.0%	5.3%	2.1%	25.1%	17.7%	0.4%	13.2%	0.4%	0.4%	12.3%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	88.583[a]	18	0.000

Continued

Likelihood Ratio	103.671	18		0.000	
Linear-by-Linear Association	17.195	1		0.000	

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	0.267	0.048	4.293	0.000[c]
Ordinal by Ordinal	Spearman Correlation	0.225	0.060	3.580	0.000[c]

Table 8. Vitamin D deficiency and dead sperm n = 243.

			dead sperm %											Total
			0.00	20.00	30.00	35.00	40.00	55.00	60.00	65.00	70.00	80.00	95.00	
vitamin D Deficiency	>more than 30 ng/dl normal	Count	11	16	8	1	17	1	2	1	20	1	1	79
		% within vitamin D Deficiency	13.9%	20.3%	10.1%	1.3%	21.5%	1.3%	2.5%	1.3%	25.3%	1.3%	1.3%	100.0%
	20 - 30 ng/dl insufficient	Count	2	0	3	0	0	0	0	0	10	2	0	17
		% within vitamin D Deficiency	11.8%	0.0%	17.6%	0.0%	0.0%	0.0%	0.0%	0.0%	58.8%	11.8%	0.0%	100.0%
	<20 ng/dl deficient	Count	40	1	21	0	0	0	0	0	26	30	29	147
		% within vitamin D Deficiency	27.2%	0.7%	14.3%	0.0%	0.0%	0.0%	0.0%	0.0%	17.7%	20.4%	19.7%	100.0%
Total		Count	53	17	32	1	17	1	2	1	56	33	30	243
		% within vitamin D Deficiency	21.8%	7.0%	13.2%	0.4%	7.0%	0.4%	0.8%	0.4%	23.0%	13.6%	12.3%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	122.850[a]	20	0.000
Likelihood Ratio	135.612	20	0.000
Linear-by-Linear Association	6.869	1	0.009

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	0.168	0.058	2.653	0.008[c]
Ordinal by Ordinal	Spearman Correlation	0.203	0.060	3.215	0.001[c]

3.9. Vitamin D and Sun Exposure

A total of 65.5% of subjects were having sun exposure less than 1 hour, 20.25 having 2 - 3 hours, 10.7% having 3 - 4 hours, 4.5% having 4 - 5 hours and 4.15 have history of sun exposure more than 5hours daily. Pearson chi square was 95.257, df 8, Asymp. sig. (2-sided) $p < 0.001$ Likely hood ratio 104.121 df 8, Asymp. sig. (2-sided) $p < 0.001$ Linear by linear association was 77.130, df 1, Asymp. sig. (2-sided) $p < 0.001$, Interval by interval Pearsons R value was −0.565, Approx. Sig $p < 0.001$. Ordinal by ordinal Spearman correlation value was −0.597, Approx. Sig $p < 0.001$ (Table 9).

Table 9. Vitamin D deficiency and sun exposure n = 243.

			sun exposure					Total
			<1 hr/day	2 - 3 hr/day	3 - 4 hr/day	4 - 5 hrs/day	>5 hrs/day	
vitamin D Deficiency	>more than 30 ng/dl normal	Count	15	28	18	9	9	79
		% within vitamin D Deficiency	19.0%	35.4%	22.8%	11.4%	11.4%	100.0%
	20 - 30 ng/dl insufficient	Count	11	5	0	0	1	17
		% within vitamin D Deficiency	64.7%	29.4%	0.0%	0.0%	5.9%	100.0%
	<20 ng/dl deficient	Count	121	16	8	2	0	147
		% within vitamin D Deficiency	82.3%	10.9%	5.4%	1.4%	.0%	100.0%
Total		Count	147	49	26	11	10	243
		% within vitamin D Deficiency	60.5%	20.2%	10.7%	4.5%	4.1%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	95.257[a]	8	0.000
Likelihood Ratio	104.121	8	0.000
Linear-by-Linear Association	77.130	1	0.000

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	−0.565	0.045	−10.618	0.000[c]
Ordinal by Ordinal	Spearman Correlation	−0.597	0.051	−11.562	0.000[c]

3.10. Vitamin D and Rapid Linear Progression of Sperms

There was strong association and significant correlation between vitamin D levels and rapid linear progression movement of sperms as shown in graph 1. As the vitamin D level increases the linear progression of sperms also increases. Rapid linear movements were more decreased in subjects within low vitamin D (**Figure 1**).

3.11. Vitamin D and Semen (Paired Statistic and Correlations)

The paired sample testing and paired samples correlation were analyzed and found statistically significant. Various means and standard deviations with standard error of mean were checked in parallel to correlations and significance as shown in the p-value was statistically significant vitamin D level with motile sperms $p < 0.001$ calcium level $p < 0.001$ rapid linear progression $p < 0.001$ slow/non linear progression $p < 0.001$ non motile sperms ($p < 0.590$), dead sperms ($p < 0.108$), normal forms of sperms $p < 0.001$, abnormal form sperms $p < 0.001$ and head abnormalities (0.239) (**Table 10**).

Table 10. Paired samples statistics and correlations of vitamin D and semen parameters n = 243.

Pair no:	variable	Mean	Std. Deviation	Std. Error Mean	Correlation	Sig.
Pair 1	Vitamin D Level	26.2025	22.06323	1.41536	0.342	0.000
	motile sperm (normal 70% - 90%)	29.7325	26.33287	1.68925		
Pair 2	Vitamin D Level	26.2025	22.06323	1.41536	0.885	0.000
	serum calcium level	8.8210	0.96446	0.06187		
Pair 3	Vitamin D Level	26.2025	22.06323	1.41536	0.232	0.000
	rapid linear progression (Normal > 25%)	2.4115	3.75095	0.24062		
Pair 4	Vitamin D Level	26.2025	22.06323	1.41536	0.399	0.000
	slow/non linear progression	12.1111	13.11929	0.84160		
Pair 5	Vitamin D Level	26.2025	22.06323	1.41536	0.035	0.590
	non progressive	61.6008	36.83593	2.36303		
Pair 6	Vitamin D Level	26.2025	22.06323	1.41536	−0.103	0.108
	dead sperm %	48.0041	33.70123	2.16193		
Pair 7	Vitamin D Level	26.2025	22.06323	1.41536	0.356	0.000
	Normal form (>4%)	49.4033	35.98602	2.30850		
Pair 8	Vitamin D Level	26.2025	22.06323	1.41536	−0.231	0.000
	abnormal sperm %	28.7860	29.04235	1.86307		
Pair 9	Vitamin D Level	26.2025	22.06323	1.41536	−0.076	0.239
	head abnormalities	2.0741	1.40346	0.09003		

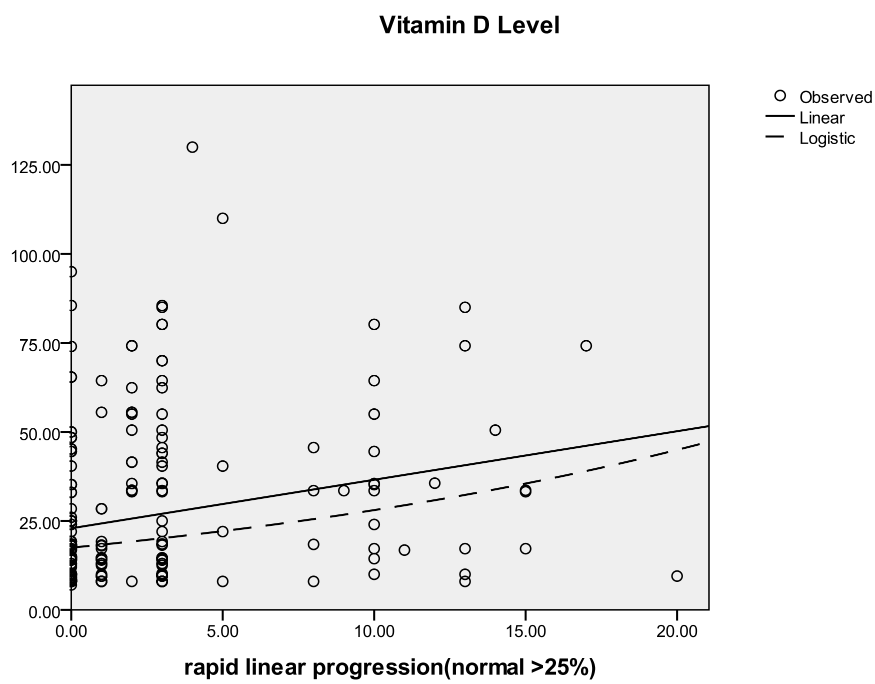

Figure 1. Linear and logistic Correlation of Vitamin D Level with Rapid Linear Progression of Sperms.

3.12. Vitamin D and Semen Parameters (Paired Sample Tests)

In relation to semen parameters and vitamin D levels paired sample test was performed with mean and SD, upper and lower limits, with 95% confidence interval as shown in Table 11, the p-value was statistically significant vitamin D level with motile sperms ($p < 0.05$), calcium level $p < 0.001$ rapid linear progression $p < 0.001$ slow/non linear progression $p < 0.001$ non motile sperms $p < 0.001$ dead sperms $p < 0.001$ normal forms of sperms $p < 0.001$ abnormal form sperms ($p < 0.319$) and head abnormalities $p < 0.001$ (Table 11).

4. Discussion

Issue of infertility is common all over the world. It is bilateral some time on male side and some time on female side and rarely both sides. They are primary and secondary. They are correctable in some situations while not in many like azoospermia. Some genetic diseases are also responsible for infertility in male and females. A social, cultural, ethical and religious value plays a great role in infertility. When a male partner is infertile his wife is not allowed for fertility with other male due to social, cultural, ethical and religious values. Simultaneously when a female is infertile the same social, cultural, ethical and religious values allows male to marry other women.

Table 11. Vitamin D deficiency and semen parameters paired samples test n = 243.

	Paired Differences						t	Sig. (2-tailed)
					95% Confidence Interval of the Difference			
	Variable pair	Mean	Std. Deviation	Std. Error Mean	Lower	Upper		
Pair 1	Vitamin D Level—motile sperm (normal 70% - 90%)	−3.53004	27.98026	1.79493	−7.06573	0.00565	−1.967	0.050
Pair 2	Vitamin D Level—serum calcium level	17.38148	21.21396	1.36088	14.70081	20.06216	12.772	0.000
Pair 3	Vitamin D Level—rapid linear progression (Normal >25%)	23.79095	21.50569	1.37959	21.07341	26.50849	17.245	0.000
Pair 4	Vitamin D Level—slow/non linear progression	14.09136	20.69175	1.32738	11.47667	16.70604	10.616	0.000
Pair 5	Vitamin D Level—non progressive	−35.39835	42.27522	2.71196	−40.74041	−30.05630	−13.053	0.000
Pair 6	Vitamin D Level—dead sperm %	−21.80165	42.14697	2.70373	−27.12749	−16.47580	−8.064	0.000
Pair 7	Vitamin D Level—normal form (>4%)	−23.20082	34.87226	2.23706	−27.60741	−18.79424	−10.371	0.000
Pair 8	Vitamin D Level—abnormal sperm %	−2.58354	40.33095	2.58723	−7.67991	2.51283	−0.999	0.319
Pair 9	Vitamin D Level—head abnormalities	24.12840	22.21380	1.42502	21.32138	26.93541	16.932	0.000

Lot of factors are responsible for infertility in males, very recently Vitamin D has been recognized as one of contributing factor in male infertility.

In current study vitamin D level were analyzed in male subjects who reported with primary infertility. The results of current study were noticeable because the vitamin D levels were below normal range in 67% of cases as compared to control subjects where it was 28%. This indicates the relationship of vitamin D and infertility.

Pakistan is a developing nation with decreased resources and deprived population growth control. Limitations in the health management system are major issues. Current study was carried out to observe the association of serum vitamin D levels in subjects with infertility. Sun exposure is sufficient around the year in most parts of the country, but concept of sunbath is negligible. In females sun exposure is limited due to parda, but males usually remain outdoors during daytime mostly for earning purpose. Usually health problems are ignored due to unsatisfactory health education [16].

Vitamin D is acquired through nutritional consumption. Ultraviolet B radiation (UV-B) from sun exposure is required for synthesis of Vitamin D in the derma. Endogenous vitamin D (cholecalciferol or D3) is biologically inactive form. UV-B rays in the skin are needed for conversion of 7-dehydroxycholesterol to cholecalciferol D3. Nutritional forms of vitamin D are D2 and D3. Fungi and yeast in the presence of UV rays are needed for synthesis of D [17].

In current study 60.5% of the subjects were having history of sun exposure less than 01 hour.

This may be the strong risk factor contributing to vitamin D deficiency and creating a bias also.

Regulation of calcium is maintained by vitamin D, for performance of reproductive functions such as hyper activation, acrosomal reaction, spermatogenesis, and sperm motility calcium is the vital component [17] [18].

The ion of calcium plays vital role in the fertilization at different levels of sperm journey from ejaculate to penetration into ovum for fertilization. Almost all the steps like rapid movement, attraction towards ovum, attachment and penetration are calcium dependent [19].

In current research the mean serum calcium was very low, which may be also additional factor for decreased motility of sperms and their rapid linear progression which was markedly decreased in current analysis.

Hypertension, cardiovascular disease, stroke, diabetes, and cancer as well as serum androgens had also been associated with vitamin D deficiency. The role of vitamin D in male reproduction is not yet resolved [20] [21]. No any difference before and after alteration of season (spring and summer) had been noted [22].

Our research supports the statement that vitamin D deficiency is also one of contributing risk factors in males with infertility.

Required concentrations of vitamin D for reproductive system and other organ systems are still not established. The role of vitamin D deficiency in decreased reproductive activity is still unclear and further studies in regard of pathogenesis and treatment in infertility are required [23].

In Pakistan no organized diet schedule had been implemented to combat the vitamin D deficiency. Lot of research is needed to sort out the effect and relation of vitamin D with fertility.

Some studies had shown a weak association between sex hormone, vitamin D and phosphorus, while in other studies no relationship was observed among vitamin D and sex hormones [24] [25]. It may be concluded from reports that the hypothalamic-pituitary-gonadal axis does not affect the outcome of different parameters of sperm in vitamin D deficient subjects, some studies also concluded no relationship of vitamin D deficiency with sperm count and concentrations [9] [25] [26].

This study only covers the relation of vitamin D and infertility, no hormonal analysis was done because of limited resources. Efforts should be done on large scale in different areas of Pakistan to confirm the correlation of vitamin D and sex hormones.

In a research (cross sectional) by Yang et al. affirmative relationship among sperm motility and vitamin D levels was observed [11].

In current research there was strong correlation of sperm motility and vitamin D levels $p \leq 0.000$

In humans elongated and round spermatids were communicated with VDR as well as enzymes that metabolize the vitamin D [8].

Calcium homeostasis influence the compromised reproductive ability of the body that may be induced by vitamin D deficiency, it indicates that the compromised reproductive ability can be corrected by supplementation of calcium and vitamin D [22].

It has been observed in current research that vitamin D has a significant correlation with calcium $p \leq 0.000$.

Consequently it had proposed that sperm motility might be affected by deficiency and/or insufficiency of vitamin D [25].

Results of current study also favors above statement that there is linear relationship of there is strong relation of sperm motility and vitamin D levels.

The role of vitamin D had been established in various organs and tissues, mostly in male reproductive activity and spermatogenesis, but the precise mechanism by which vitamin D effects this is still unclear [27].

The results of present study concluded that infertile males are vitamin D deficient as compared to controls.

The optimum sperm ability may directly depend on the consequence of vitamin D, but the indirect influence of calcium homeostasis on impaired infertility as observed in animal models was restored after correction of serum calcium levels [28] [29] [30].

In present study the rapid linear movement of sperms was markedly lower in subjects with infertility $p < 0.000$ which was statistically significant.

Sperm count was observed below the WHO recommended levels in 20% of young subjects and 40% of subjects had sperm concentrations below the optimal level considered for fertility [22].

The total sperm count was 00.00 to 120million but mean sperm count was 25.91 million which was below fertile levels in our subjects.

Sh. Abbasihormozi *et al.* noted a substantial association among sperm motility, and serum levels of calcium and vitamin D in OAT (oligo-astheno-teratozoospermia) group [22].

In present study the abnormal sperm percentage was with mean level 28.78 with SD29.04, this was also matchable with other studies. There was statistically significant association and relationship among sperm count, motility, morphology, serum calcium and vitamin D levels.

5. Limitations

This is a small study representing a local population.

The mass results of vitamin D levels are not available on large scale as reference from population defined. Few studies are available in relation to semen and vitamin D worldwide. Our setup lacks that.

Local temperature is very high sometimes at world record levels here. So it affects the sperm count and viability.

Root level causes of infertility are not searched as reference here, so we can search on large scale.

It is hard to share the reports for research purpose especially semen analyses because of social, cultural and religious values.

6. Conclusion

Vitamin D3 levels are low in infertile males and are associated with semen parameter abnormalities significantly affecting the male fertility. It does not only affect the count of sperm but morphology, chemistry and function of sperms. Low vitamin D3 levels are associated with abnormal movements of sperms with decrease rapid linear progression, slow or no progression leading to infertility in males.

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Conflict of Interests

The authors declare no conflict of interest for this study.

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Contribution of Authors

Jamali AA, Tanwani BM and Jamali GM planned the current study, also had contribution in all aspects for research as data gathering, scrutiny, explanation and in writing of the document. Other authors took part in the data gathering. The study was supervised by Jamali AA. The manuscript was checked and approved by all writers.

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