Association of vitamin D status with semen parameters and male reproductive hormones in Egyptian infertile men

Original Article

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ABSTRACT

Background: Although vitamin D receptor is expressed in human sperm, little is known about the role of vitamin D (Vit. D) in male reproduction.

Aim: To assess Vit. D levels both in serum and seminal fluid and to establish the relation between serum and seminal Vit. D levels, semen parameters, male sex hormones, and serum calcium level in Egyptian infertile men.

Patients and Methods: We conducted a prospective case–control study that included 60 male patients with infertility of unknown etiology and 30 healthy fertile males as controls. They were randomly recruited from the Andrology Clinic of Assiut University Hospital (AUH) during the period from January 2018 to February 2019. Semen samples were collected and semen parameters were evaluated. Also, seminal Vit. D level was measured. Blood samples were taken to estimate serum levels of Vit. D, calcium, testosterone, luteinizing hormone, and follicle-stimulating hormone.

Results: There was significant decrease of both serum and seminal Vit. D level in male infertility groups compared with the control group. A significant positive correlation was found between serum and seminal Vit. D levels in the different studied groups. Also, there was significant positive correlation between serum Vit. D level and nonprogressive sperm motility.

Conclusion: There was significant decrease of both serum and seminal Vit. D levels in Egyptian infertile males compared with controls and significant positive correlation between serum Vit. D level and nonprogressive sperm motility, suggesting the role of Vit. D in male fertility status, which requires further studies.

Key Words: Male infertility, reproductive hormones, semen parameters, vitamin D.

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INTRODUCTION

Infertility with failure to achieve a normal pregnancy after 1 year or more of unprotected and regular sexual life is prescribed in 15% of couples where about 50% of the cases are partially or wholly attributable to a male factor[1–3]. Male infertility, either primary or secondary, may be attributed to identifiable hormonal or anatomical causes[4,5]. Normal functioning of the hypothalamus, pituitary glands, and testes is required for male fertility, and complete male germ cell maturation is dependent on the balanced hormonal secretion of these glands[6,7]. Hypogonadotrophic hypogonadism, which is the failure of the pituitary gland to secrete adequate amounts of follicle-stimulating hormone(FSH) and luteinizing hormone(LH), can lead to decreased sperm count and male infertility[8]. In addition, in oligospermic men, hyperprolactinemia may inhibit pulsatile secretion of the gonadotrophin-releasing hormone with subsequent decreased pulsatile release of FSH, LH, and testosterone. It leads to secondary hypogonadism and male infertility[9]. However, the exact etiology of male infertility remains unknown in about 30–50% of infertile patients, either in those with normal semen parameters, unexplained male infertility, or in others with abnormal semen parameters who are categorized as having idiopathic male infertility[10–12].

Although vitamin D (Vit. D) is most strictly connected with regulation of calcium and bone homeostasis[13], it has been suggested to have many other biological actions, including effects on the immune system, diabetes, and cancer prevention[14,15]. One of the recently identified target zones of Vit. D is male reproductive function. The basicsof the interplay between Vit. D and reproduction is best reflected by the expression of vitaminD receptors(VDR) and the metabolic enzymes(CYP2R1, CYP27B1, and CYP24A1) in the different tissues of both sex reproductive systems[16]. VDR was shown to be expressed in the neck and post-acrosomal regions of the sperm whilethe metabolizing enzymes are expressed in the testis, epididymis, seminal

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vesicle, prostate, and spermatozoa, suggesting a local regulation of active Vit. D, which may be implicated in spermatogenesis andsperm function[17,18].

The exact role of VDR in the sperm nucleus is not known. It may act as a protective genomic factor, as it is essential for the proper control of sperm DNA integrity and maintenance of genome stability[19].Vit. D could decrease early apoptosis and necrosis, and increase sperm motility in asthenozoospermia[20,21]. It has been suggested that Vit. D increases intracellular calcium concentration with a positive impact on sperm motility, and induces acrosome reaction in mature sperm from healthy males[18,22].Vit. D might also enhance sperm motility by promoting the synthesis of ATP, both through the cAMP/PKA pathway and the increase in intracellular calcium ions[23,24].

Previous researchers have suggested a positive relationship between the semen levels of Vit. D and abnormal semen parameters[18–22]. However, the interrelationship between serum and seminal levels of Vit. D, semen parameters, and male sex hormones is still unclear.

Our study was conducted to establish the relationship between serum and seminal Vit. D levels, semen parameters, male reproductive hormones, and serum calcium level in Egyptian infertile men. In addition, we aimed to clarify if serum Vit. D level is a reflection of its seminal level or not.

PATIENTS AND METHODS

Study design and population

A prospective case–control study was performed at the Dermatology, Venereology and Andrology Department and Medical Biochemistry Department, Faculty of Medicine, Assiut University, Assiut, Egypt. The study was approved by the Medical Ethics Committee of Faculty of Medicine, Assiut University (Approval number IRB17100087) and was carried out in accordance with the guidelines of the Helsinki Declaration. An informed written consent was obtained from all participants before the study. Sample size calculation was conducted using theG Power program to detect the significant difference in semen and serum Vit. Din groups under the study with power 0.95 in a hypothetical effect size of 0.33.

A total of 246 infertile male patients who attended the Andrology Outpatient Clinic of Assiut University Hospital (AUH)from the beginning of January 2018 to the end of February 2019were screened. After excluding 186 patients who did not fulfill the inclusion criteria, a total of 60 male patients with infertility of unknown etiology and 30 healthy fertile males (as controls) were recruited and incorporated into the study.

Inclusion criteria included infertile male patients without any detectable cause of infertility with normal clinical examination ±normal semen parameters.

Exclusion criteria

The study excluded patients with any apparent physical finding and any known pathology of the reproductive tract (e.g. prostatitis, epididymitis, any genital tract infections, varicocele, diminished testicular volume or abnormal hormonal profile, etc.), or those who received Vit. D therapy or other hormonal therapies. Moreover, patients with BMI more than24.9, chronic systemic disease, cancer, malabsorption, poor general status or combined male and female factor of infertility were also excluded.

The participants were subdivided into three groups:

(1) Control group: included 30 fertile males.
(2) Unexplained male infertility group: included 30 infertile male patients with normal clinical examination and normal semen parameters.
(3) Idiopathic male infertility group: included 30 infertile male patients presented with normal clinical examination and abnormal semen parameters.

A detailed history was taken from all patients including:personal, sexual, medical history, and family history in addition to the fertility history of his wife. General physical examination and genital examination of testis, epididymis, vas deferens, spermatic cord, penis, scrotum, and inguinal lymph nodes were performed.

Semen analysis

After abstinence period of 2 to7 days, the semen sample of each patient was obtained by masturbation into a sterile plastic container. Patients were instructed to report any semen loss during semen collection. After complete liquefaction in a 37°C incubator, semen samples were evaluated for physical criteria and then centrifuged where the pellets are used to assess the semen parameters based on the World Health Organization (WHO) laboratory guidelines 2010 as a reference[2]. Normal sperm parameters are considered if the total sperm count more than 39 million sperm per ejaculate, sperm concentration more than 15 million sperm per ml, progressive motility more than 32%, total motility more than 40%, and normal morphology more than or equal to 4%. Seminal fluidwas evaluated for Vit. D levelas described in the kit manual supplied by Epitope Diagnostics Inc.(San Diego, California, USA) (EDITM Total 25-OHVit. D EIA Kit, Cat No.: KT715, USA).

Biochemical analysis

Venous blood samples from all patients were collected via venipuncture of superficial vessels in the antecubital fossa or hands by a well-trained clinician. Five milliliters of peripheral venous blood was withdrawn from each patient; it was dispensed into plain tubes and then centrifuged for 15min at aspeed of 2000–3000 rpm and the serum was collected. Serum is divided into five Eppendorf tubes and then stored at −20°C to avoid repeated freeze–thaw cycles till batch assay of our markers. Serum level of total Vit. D was measured using the same kit as seminal levels

(EDITM total 25-OH Vit. D EIA Kit, Cat No.: KT715, USA). Serum calcium was measured by colorimetric method using Abcam assay kit (ab102505, Cambridge, United Kingdome). FSH and LH levels were measured by enzyme-linked immunosorbent assay kits supplied by Perfecta Ease Biotec (Beijing) Co., China, Cat No.:10001 and 10004, respectively. Testosterone hormone levels were detected bythe enzyme-linked immunosorbent assay kit supplied by Abia (AB Diagnostic System, Berlin, German; Cat No.: DK.004.01.3).

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS version 19; SPSS Inc., Chicago, Illinois, USA) software program. Data statistically stated in terms of mean, SD, median, range, frequencies (number of cases), and relative frequencies (percentages) when appropriate. χ^2 test was used to compare between qualitative variables. Independent samples *t* test was used to compare quantitative variables between two groups in case of parametric data and Mann–Whitney test was used for nonparametric data. Spearman correlation was used to measure the correlation between quantitative variables. A *P* value less than or equal to 0.05 was considered statistically significant.

Determination of the threshold value for optimal sensitivity and specificity of our markers was done by the receiver-operating characteristic (ROC) curve, which was plotted by calculating sensitivity and specificity at multiple cutoff points. Area under the curve (AUC) of the ROC plots was calculated using discriminate analysis, where AUC=1.0 means perfect separation of test values into two groups and AUC=0.5 means no distributional differences. AUC more than 0.8 indicates excellent discriminating power of the test and AUC more than 0.7 indicates a discriminating strength of statistical significance.

RESULTS

The demographic data of the study

The demographic data of the studied groups are summarized in Table 1. A total of 60 infertile male patients of unknown etiology were included in the study and were divided into two groups as mentioned before. There was no statistically significant difference between our studied groups regarding the mean of age and smoking. Smoking is a prevalent habit in Upper Egypt and we tried to select some non-heavy smokers into our study as we found a great difficulty to exclude all smokers. Both types of unexplained and idiopathic infertility were more common in smokers, rural residents, and workers (Table 1).

A statistically significant difference was found between the two infertility patients' groups regarding the duration of infertility as the mean of disease duration ±SD in the idiopathic male infertility group was 4.13±1.91 and the median was 4 with range 1–8 years and suffered from a longer period of infertility in comparison to the unexplained infertility group; the mean of disease duration ±SD was 1.95±0.83 and the median was 2 with range 1–4 years(*P*=0.000).

Table 1: Demographic data of the studied participants

	Control [n (%)]	Unexplained infertility [n (%)]	Idiopathic infertility[n (%)]	*P* value[1]	*P* value[2]	*P* value[3]
Age (years)						
Mean ±SD	28.70±3.71	29.93±4.39	29.20±5.27	0.245	0.679	0.571
Median (range)	28 (23–39)	30 (19–37)	30 (24–39)			
Smoking						
Smoker	14 (46.7)	18 (60.0)	16 (53.3)	0.301	0.07	0.091
Nonsmoker	16 (53.3)	12 (40.0)	14 (46.7)			
Residence						
Rural	19 (63.3)	22 (73.3)	26 (86.7)	0.405	0.037*	0.197
Urban	11 (36.7)	8 (26.7)	4 (13.3)			
Occupation						
Employee	13 (43.3)	5 (16.7)	0	0.076	0.000*	0.014*
Farmer	1 (3.3)	1 (3.3)	6 (20.0)			
Worker	16 (53.3)	24 (80.0)	24 (80.0)			

**P* value less than or equal to 0.05 is significant.
*P*1: unexplained infertility versus control.
*P*2: idiopathic infertility versus control.
*P*3: unexplained infertility versus idiopathic infertility.

Characteristics of participants' semen analysis

Table 2 showed a significant decrease of semen volume in the idiopathic male infertility group compared with both control and unexplained male infertility group (P=0.000). The two male infertility groups showed a significant decrease in sperm count and concentration compared with the control group, where the idiopathic male infertility group has a lower sperm count and concentration compared with the unexplained male infertility group. As regards sperm motility (progressive and nonprogressive), there was a significant decrease in both infertility groups compared with the control group and also in the idiopathic male infertility group compared with the unexplained infertility group. In addition, the idiopathic male infertility group expressed a significant decrease in sperm morphology compared with both control and unexplained male infertility group (P=0.000).

Characteristics of participants' hormonal profile

The reproductive hormonal profile revealed no significance among the three groups except for testosterone, where its levels showed a significant decrease in the unexplained male infertility group compared with the control group (*P*=0.005). Serum calcium levels were almost equal in different study groups. There was significant decrease in serum and seminal Vit. D level in both unexplained and idiopathic male infertility group compared with the control group (*P*=0.008, 0.001, .000, 0.0000, respectively) as shown in Table 3. Moreover, seminal Vit. D level was significantly lower in idiopathic male infertility in comparison to the unexplained group, which is shown in Table 3.

Table 2: Semen parameters in different study groups

	Control	Unexplained infertility	Idiopathic infertility	*P* value[1]	*P* value[2]	*P* value[3]
Volume (ml)						
Mean ±SD	3.10±0.81	2.98±0.99	2.27±0.55	0.494	0.000*	0.003*
Median (range)	3.0 (2.0–4.0)	3.0 (1.5–5.0)	2.0 (1.5–3.0)			
Sperm concentration (10^6/ml)						
Mean ±SD	66.13±21.10	48.13±20.96	16.93±15.80	0.002*	0.000*	0.000*
Median (range)	70 (28–100)	45 (20–90)	11 (1–45)			
Sperm count (10^6/ejaculate)						
Mean ±SD	207.33±94.15	145.10±93.06	38.70±34.00	0.001*	0.000*	0.000*
Median (range)	192 (70–400)	120 (40–400)	31.5 (1.5–95)			
Rapid linear progressive motility						
Mean ±SD	22.33±9.07	16.83±8.35	2.07±3.34	0.028*	0.000*	0.000*
Median (range)	20.0 (10.0–40.0)	17.5 (5.0–30.0)	0.0 (0.0–10.0)			
Slow and nonlinear progressive motility						
Mean ±SD	35.83±9.57	30.50±8.34	14.23±7.31	0.017*	0.000*	0.000*
Median (range)	37.5 (15.0–50.0)	27.5 (20.0–50.0)	15.0 (0.0–25.0)			
Progressive motility						
Mean ±SD	58.17±9.42	47.33±10.89	16.30±8.12	0.000*	0.000*	0.000*
Median (range)	55.0 (40.0–70.0)	45.0 (35.0–70.0)	15.0 (0.0–30.0)			
Nonprogressive motility						
Mean ±SD	22.83±5.20	25.83±8.21	10.40±5.11	0.084	0.000*	0.000*
Median (range)	22.5 (15.0–30.0)	25.0 (10.0–40.0)	10.0 (1.0–20.0)			
Total motility						
Mean ±SD	81.00±8.75	73.17±12.00	26.70±10.18	0.010*	0.000*	0.000*
Median (range)	80.0 (65.0–95.0)	75.0 (50.0–90.0)	30.0 (3.0–35.0)			
Immotile sperms						
Mean ±SD	18.83±8.78	26.83±12.00	73.30±10.18	0.008*	0.000*	0.000*
Median (range)	17.5 (5.0–35.0)	25.0 (10.0–50.0)	70.0 (65.0–97.0)			
Sperm morphology						
Mean ±SD	5.83±1.39	5.37±1.22	2.20±1.13	0.191	0.000*	0.000*
Median (range)	6 (4–8)	5 (4–8)	2 (1–5)			

*P valueless than or equal to 0.05 is significant, $P1$: unexplained infertility versus control, $P2$: idiopathic infertility versus control, $P3$: unexplained infertility versus idiopathic infertility.

Table 3: Distribution of serum levels of male reproductive hormonal, serum calcium level, and serum and seminal vitamin D levels in different study groups

	Control	Unexplained infertility	Idiopathic infertility	*P* value[1]	*P* value[2]	*P* value[3]
FSH (mIU/ml)						
Mean ±SD	5.76±1.18	6.26±2.74	6.07±2.41	0.888	0.615	0.745
Median (range)	5.5 (4.1–8.2)	5.3 (4.0–15.3)	5.3 (3.9–13.9)			
LH (mIU/ml)						
Mean ±SD	6.36±1.25	6.40±0.78	6.26±0.95	0.455	0.918	0.231
Median (range)	6.2 (4.9–9.8)	6.2 (5.0–8.9)	6.0 (4.5–8.6)			
Testosterone (ng/dl)						
Mean ±SD	491.67±83.02	431.29±62.52	453.29±56.69	0.005*	0.085	0.217
Median (range)	474 (322–668)	422 (285–523)	460 (301–589)			
Calcium (mg/dl)						
Mean ±SD	7.39±0.72	7.30±0.76	7.37±0.75	0.433	0.446	0.663
Range	5.1–8.5	6.3–9.0	6.2–9.1			
Serum vitamin D (ng/ml)						
Mean ±SD	24.08±4.58	21.46±3.90	20.24±4.57	0.008*	0.001*	0.188
Range	8.9–30.4	16.2–31.6	14.6–30.2			
Seminal vitamin D (ng/ml)						
Mean ±SD	11.23±1.30	8.39±1.51	7.31±1.33	0.000*	0.000*	0.003*
Range	8.4–13.9	5.9–10.9	5.6–10.1			

FSH, follicle-stimulating hormone; LH, luteinizing hormone.
**P* valueless than or equal to 0.05 is significant.
*P*1: unexplained infertility versus control.
*P*2: idiopathic infertility versus control.
*P*3: unexplained infertility versus idiopathic infertility.

Correlations between serum and seminal Vit. D levels, semen parameters, male reproductive hormones, and serum calcium level in different groups

A significant positive correlation was found between serum and seminal Vit. D levels in unexplained and idiopathic male infertility groups (r=0.607, *P*=0.000 and r=0.931, *P*=0.000, respectively) (Table 4).

In the unexplained male infertility group, a significant positive correlation was established between serum and seminal Vit. D levels and serum calcium level (r=0.482, *P*=0.007 and r=0.477, *P*=0.008, respectively) (Table 5).

Our study revealed in the idiopathic male infertility group, a significant positive correlation between serum Vit. D level and nonprogressive sperm motility (r=0.450, *P*=0.012). Significant negative correlations between serum and seminal Vit. D levels and serum levels of FSH and LH were found (Table 5).

ROC curve (Fig.1 and Table 6) showed serum Vit. D level at a cutoff point less than or equal to 23.61ng/ml has 76.67% sensitivity and 63.33% specificity for the prediction of unexplained male infertility with the AUC being 0.699. Also, Vit. D levels at a cutoff point less than or equal to 21.96ng/ml has 70.00% sensitivity and 76.67% specificity for the prediction of idiopathic male infertility with an AUC of 0.748. As well, serum levels of Vit. D at a cutoff point less than or equal to 22.08ng/ml has 65.00% sensitivity and 76.67% specificity for the prediction of male infertility either idiopathic or unexplained with an AUC of 0.723.

Table 4: Correlations between serum and seminal vitamin D levels in different study groups

Serum vitamin D	Seminal vitamin D		
	Control	Unexplained infertility	Idiopathic infertility
r value	0.870	0.607	0.931
P value	0.000*	0.000*	0.000*

**P* value less than or equal to 0.05 is significant.

Table 5: Correlations between serum and seminal vitamin D levels, semen parameters, male reproductive hormones, and serum calcium level in different groups

	Control group				Unexplained group				Idiopathic group			
	Serum vitamin D		Seminal vitamin D		Serum vitamin D		Serum vitamin D		Seminal vitamin D		Seminal vitamin D	
	r value	*P* value	r value	*P* value	r value	*P* value	r value	*P* value	r value	*P* value	r value	*P* value
Volume (ml)	–0.033	0.863	–0.029	0.880	0.286	0.126	–0.206	0.275	0.214	0.256	0.143	0.452
Sperm concentration (106/ml)	–0.125	0.510	–0.108	0.569	0.053	0.783	0.017	0.929	0.148	0.436	0.010	0.958
Sperm count/ejaculate (106/ejaculate)	–0.049	0.795	–0.087	0.649	0.097	0.611	0.172	0.362	0.274	0.143	0.125	0.511
Rapid linear progressive motility	0.310	0.095	0.232	0.217	–0.085	0.657	–0.134	0.480	–0.173	0.147	–0.090	0.636
Slow and nonlinear progressive motility	–0.269	0.151	–0.171	0.366	0.327	0.078	0.166	0.381	0.244	0.193	0.126	0.507
Progressive motility	0.043	0.820	0.050	0.794	0.110	0.561	0.024	0.899	0.073	0.703	0.076	0.689
Nonprogressive motility	0.128	0.502	0.072	0.707	0.053	0.783	0.102	0.591	0.450	0.012*	0.175	0.354
Total motility	0.088	0.644	0.096	0.613	0.216	0.252	0.092	0.630	0.243	0.196	0.149	0.433
Immotile sperms	–0.106	0.577	–0.100	0.599	–0.216	0.252	–0.092	0.630	–0.243	0.196	–0.149	0.433
Sperm morphology	0.032	0.866	–0.045	0.814	0.195	0.301	0.097	0.610	0.028	0.883	–0.059	0.755
FSH (mIU/ml)	0.097	0.610	0.173	0.360	0.077	0.687	–0.191	0.311	–0.406	0.026*	–0.356	0.05*
LH (mIU/ml)	–0.095	0.619	0.163	0.388	–0.337	0.069	–0.217	0.249	–0.388	0.034*	–0.430	0.018*
Testosterone (ng/dl)	0.063	0.742	0.114	0.549	0.180	0.342	0.179	0.344	–0.160	0.400	–0.206	0.274
Calcium (mg/dl)	–0.243	0.196	–0.204	0.280	0.482	0.007*	0.477	0.008*	0.282	0.131	0.192	0.308

FSH, follicle-stimulating hormone; LH, luteinizing hormone; r, Spearman's correlation coefficient; *Pvalueless than or equal to 0.05 is significant.

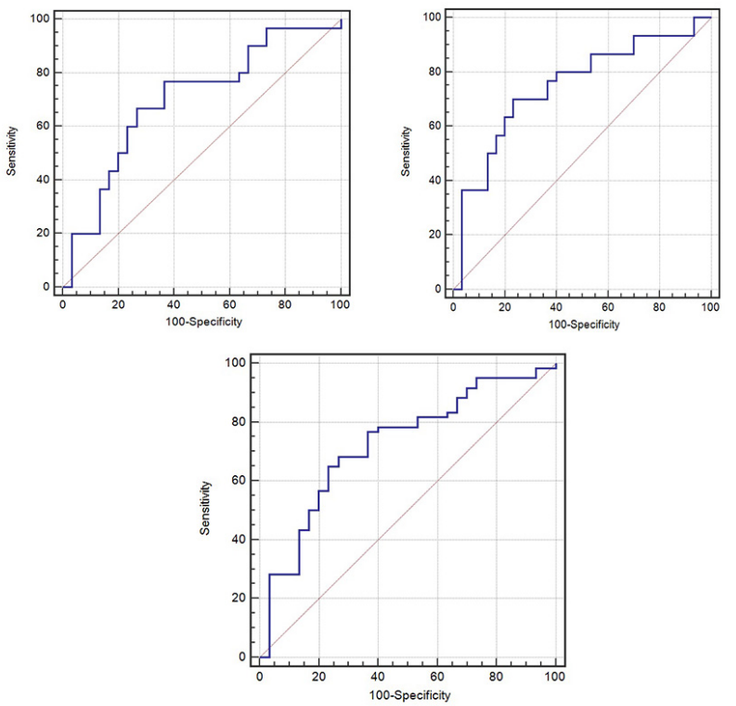

Fig. 1: ROC curve to distinguish the cutoff value of serum vitamin D of unexplained infertility, idiopathic infertility, and infertility as a whole. ROC, receiver-operating characteristic.

Table 6: Performance characteristics of serum vitamin D for detection of unexplained infertility, idiopathic infertility, and infertility as a whole

Group	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
Unexplained infertility	≤23.61ng/ml	76.67	63.33	67.6	73.1	70.00	0.699
Idiopathic infertility	≤21.96ng/ml	70.00	76.67	75.0	71.9	71.67	0.748
Infertility	≤22.08ng/ml	65.00	76.67	84.8	52.3	68.89	0.723

PPV, Positive predictive value; NPV, Negative predictive value; AUC, Area under the curve.

DISCUSSION

Infertility harbors massive psychosocial burdens among people and is one of the major stressors according to a study by Patel *et al.*[25]. Pandemic Vit. D deficiency reports have extended the spectrum of extra-skeletal research on Vit. D,where the male reproductive function is an important area of interest[26].

In our study, we searched Vit. D levels (both serum and seminal) in infertile men attending the Andrology Clinic of AUH during a specific period of time and analyzed its relation to semen parameters, serum calcium level, and the levels of male reproductive hormones in our community.

Among different populations, the level of Vit. D varies because of the variations in sun exposure, diet, clothing style, and skin color[27].

In tropical countries, the prevalence of serum Vit. D deficiency has a percentage of between 30 and 93%. It is considered one of the major health problems in both developed and developing countries[28].

The current study revealed a significant decrease in both serum and seminal Vit. D in Egyptian infertile males compared with the healthy fertile control group, as VDR

is found in male reproductive tissues such as the testes, ejaculatory tract, and mature spermatozoa[16,17], and this indicates a role of Vit. D in male reproduction and fertility.

Our results are in agreement with the results of Abbasihormozi *et al.*[26] who found a significant prevalence of Vit. Ddeficiency and insufficiency in the Iranian subfertile men suggesting that Vit. D deficiency may be associated with male infertility. The same was found in another study performed by Alzoubi *et al.*[29], who found that patients with idiopathic male infertility showed significantly lower serum levels of Vit. D at baseline when compared with controls.

Moreover, a previous study revealed a significant decrease in Vit. D levels in patients that had altered sperm parameters[30]. Also, Zhu *et al.*[31] revealed that the levels of serum Vit. D were significantly lower in asthenozoospermia, oligoasthenozoospermia, and azoospermia Chinese infertile patients than those in fertile men.

In addition, the role of Vit. D in male sex hormone production is still unclear. Some previous studies have shown a positive correlation between serum Vit. D and total testosterone levels[32,33]. Our results showed no significant associations among serum Vit. D and testosterone levels in the studied groups. This was in agreement with a previous study which reported that the serum level of Vit. D didnot correlate withtotal testosterone levels in Korean men even after adjustment for confounding variables[34]. Also, Tirabassi *et al.*[35] showedno association between Vit. D and testosterone levels. Moreover, Lerchbaum *et al.*[36] found no significant effect of Vit. D supplementation on serum total testosterone levels in middle-aged healthy men with low total testosterone levels. Besides, Heijboer *et al.*[37] observed that Vit. D supplementation caused a clear increase in Vit. D levels without any statistically significant effect on testosterone levels. This suggests that Vit. D treatment has no relevant effect on serum testosterone levels in men.

As regards, FSH and LH hormones, our data didnot reveal any significant correlations between serum and seminal Vit. D levels and FSH and LH serum levels in the unexplained male infertility group. However, serum and seminal Vit. D levels had significant negative correlations with both FSH and LH serum levels only in the group of idiopathic male infertility. This was in agreement with a previous study which concluded that serum FSH and LH measures didnot differ significantly across categories of serum Vit. D concentrations among young Spanish males[38]. A previous study by Rehman *et al.*[30] revealed that only serum LH level had a nonsignificant negative correlation with Vit. Dlevel while serum FSH level didnot. In this group of men referred for unexplained infertility, Vit. D is not correlated with total testosterone level; however, it is negatively correlated with LH level. As LH regulates the secretion of testosterone, these findings may be explained by the fact that low Vit. D levels may suppress testicular production of testosterone that is corrected by the overproduction of LH.

In addition, we didnot find any significant correlations between serum Vit. D level and sperm count or concentration in all studied groups. This is in accordance with a previous study[39] that showed that there was no significant difference between semen parameters in both fertile and infertile groups with and without Vit. D deficiency. On the contrary, another study performed on infertile men found a positive correlation between serum Vit. D level with both sperm concentration and progressive sperm motility, which significantly improved afterVit. D treatment for 6 months[40].

In addition, a previous Turkish study was performed on infertile men who showed that Vit. D insufficiency was common among the infertile men and there was a positive correlation between Vit. D and sperm concentration. Vit. D supplementation may be the appropriate treatment for infertile men with documented Vit. D deficiency[41].

Previous researches have been conducted to evaluate the association of Vit. D status and sperm motility and morphology with contrary results. Our results revealed a significant positive correlation between serum Vit. D level and nonprogressive sperm motility in the idiopathic male infertility group while no associations were found between serum Vit. D level and sperm morphology in all the studied groups. Azizi *et al.*[42] declared that total sperm motility positively correlated with a serum level of 25OH Vit. D in both normozoospermic and oligoasthenoteratozoospermic men. Abbasihormozi *et al.*[26] revealed that Vit. D levels showed no correlation with semen parameters and hormonal profiles in normospermic men. However, sperm motility and calcium positively correlated with Vit. Din the oligoasthenoteratozoospermic group. Another study showed a statistically significant correlation between baseline serum Vit. D level and all semen parameters. After treatment with Vit. D, there was a statistically significant improvement in sperm progressive forward motility and total sperm motility after Vit. D restoration[30]. In addition, a previous study reported that the incidence of hypovitaminosis D among men seeking fertility therapy is similar to the national average, and oral Vit. D treatment improves sperm motility among this men[43].

LIMITATION OF THE STUDY

The limitations of our study were the small sample and serum phosphorus and parathyroid hormone levels were not estimated. Furthermore, our study didnot assess the seasonal variations of Vit. D levels among different groups. We recommend further Egyptian studies on a larger sample of participants to prove or disprove our assumption.

CONCLUSION

The significant decrease in both serum and seminal

Vit. D levels in Egyptian infertile males compared with controls and the significant positive correlation between serum Vit. D level and nonprogressive sperm motility suggest the role of Vit. D in male fertility status. However, there were no correlations between serum levels of Vit. D and male reproductive hormones, indicating that the effect of Vit. D on male fertility may not be mediated through male reproductive hormones.

CONFILCT OF INTEREST

There are no conflicts of interest.

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