Study of vitamin D status in male patients with hypogonadism

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Background
Male hypogonadism is a clinical and biochemical syndrome that results from failure to produce adequate testosterone levels, normal sperm count, or both. Male hypogonadism may adversely affect multiple organ functions and quality of life. Vitamin D is a steroid hormone; the major action of vitamin D is linked to maintaining musculoskeletal health. However, several epidemiological studies have suggested extraskeletal benefits of vitamin D. There is an accumulating body of evidence which suggests that vitamin D is involved in reproductive and gonadal functions. Although some studies have demonstrated that vitamin D levels are positively associated with androgen levels and that vitamin D supplementation may increase testosterone levels, other studies have observed a U-shaped association of vitamin D and hypogonadism in middle-aged men, in contrast, other studies fail to find an association between vitamin D and testosterone especially in young healthy men after exclusion of other confounding factors. Thus, the aim of our study was to study the possible association of vitamin D status with male hypogonadism among different age groups.

Patients and methods
The study included 80 men. Group I included 40 male patients aged 20 to less than 45 years who were further subdivided into two subgroups: group Ia included 20 male patients diagnosed with hypogonadism and group Ib included 20 eugonadal men serving as a control. Group II: included 40 male patients aged 45–70 years subdivided into two subgroups: group IIa included 20 male patients diagnosed with hypogonadism and group IIb included eugonadal men serving as a control. Using enzyme-linked fluorescent assay technique, serum total testosterone (TT), 25-hydroxyvitamin D_3 [25(OH)D_3], luteinizing hormone, follicle stimulating hormone, estradiol, and prolactin were assessed for all enrolled individuals. Sex-hormone-binding globulin (SHBG) was assessed using the electrochemiluminescence immunoassay technique. Free androgen index (FAI) was calculated using the equation [100×TT (nmol/l)/SHBG (nmol/l)].

Results
It was found that TT, FAI, and SHBG were lower in hypogonadal men versus eugonadal men in both groups, there was no significant statistical difference between hypogonadal men in groups I and II as regards TT and SHBG (P=0.708, 0.124, respectively), whereas FAI was found to be significantly statistically lower in hypogonadal men aged 45–70 years as compared with hypogonadal men aged 20 to less than 45 years (P=0.021). There was a high prevalence of vitamin D deficiency and insufficiency in both hypogonadal and eugonadal men in the four studied subgroups. 25(OH)D_3 was not statistically different between subgroups in both groups (P=0.681, 0.823, respectively), whereas 25(OH)D_3 was significantly higher in hypogonadal men in group II versus hypogonadal men in group I (P=0.037). 25(OH)D_3 was found to be positively correlated with TT, FAI, and SHBG, but not to serum estradiol, prolactin. Correlation of 25(OH)D_3 was stronger in hypogonadal men aged 45–70 years (P=0.001) as compared with hypogonadal men aged from 20 to less than 45 years (P=0.023).

Conclusion
Low vitamin D is associated with male hypogonadism across all age groups. This association is more pronounced in elderly hypogonadal men with vitamin D deficiency.

Keywords:
Vit D, Male, Hypogonadism

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Introduction

Male hypogonadism is a clinical and biochemical syndrome that results from failure to produce adequate testosterone levels, normal sperm count, or both [1,2]. It arises from disruption of the hypothalamic–pituitary–gonadal axis at any level. Accordingly, male hypogonadism is classified according to the level of affection into either primary or secondary, both of which may arise from several congenital or acquired disorders [3].

Male hypogonadism may adversely affect multiple organ functions and quality of life [1]. It involves a complex of signs and symptoms, including increased BMI – with visceral weight gain in particular – low bone mineral density, irritability, and sexual dysfunction in the form of decreased sexual desire and function [4,5]. Reduced energy and increased fatigue are the most common presenting complaints. Male hypogonadism is associated with increased risk of osteoporosis, metabolic syndrome, cardiovascular diseases, and mood disturbances [6].

Despite the increased appreciation of its clinical significance, diagnosis of male hypogonadism is still underestimated. The incidence of hypogonadism increases slightly with age [7]. In middle-aged men, the incidence of biochemical hypogonadism varies from 2.1 to 12.8% [8]. The incidence of low testosterone and symptoms of hypogonadism in men aged 40–79 years varies from 2.1 to 5.7% [8,9].

Vitamin D is a steroid hormone, and the main status of vitamin D is mainly derived from sun exposure where ultraviolet rays induce vitamin D production in the skin. In contrast, vitamin D intake by nutrition and supplements play a minor role in determining vitamin D pool [10]. Vitamin D obtained from diet, supplement use, and sun exposure is primarily hydroxylated in the liver to 25-hydroxyvitamin D_3 [$25(OH)D_3$], then further hydroxylated to its active form, 1,25-dihydroxyvitamin D [$1,25(OH)2D_3$] in the kidney. $25(OH)D_3$ is the primary form of circulating vitamin D and considered as the best indicator to determine vitamin D status [11].

The vitamin D receptor (VDR) mediates biological actions of vitamin D. It has been found that VDR is almost ubiquitously expressed in human cells, which underlines the clinical significance of the vitamin D endocrine system [12]. The major action of vitamin D is linked to maintaining musculoskeletal health as well as calcium and phosphorous homeostasis by ensuring maximum absorption of calcium and phosphorus from the gut [13]. However, several epidemiological studies have suggested extraskeletal benefits of vitamin D, where lower levels of vitamin D are linked to several diseases namely certain cancers, autoimmune diseases, infectious diseases, type 2 diabetes mellitus, neurocognitive disorders, and infertility [13,14].

Interestingly, there is an accumulating body of evidence which suggests that vitamin D is involved in reproductive and gonadal functions [12]. The basis of this link is derived from the presence of both VDR and 1α-hydroxylase (CYP27B1) enzyme in reproductive organs. In men, VDRs are present in the testis, epididymis, prostate, and seminal vesicles [15]. Moreover, it has been suggested that vitamin D metabolism and androgen metabolism are tightly related. It has been shown that androgens increase 1-α-hydroxylase. Furthermore, it has been demonstrated that the regulation of the gene expression by vitamin D metabolites is modified according to androgen levels [12,16].

Several observational and interventional studies have demonstrated that vitamin D levels are positively associated with androgen levels [11,17] and that vitamin D supplementation may increase testosterone levels [18]. Other studies observed a U-shaped association between vitamin D and hypogonadism in middle-aged men. In contrast, other studies fail to find an association between vitamin D and testosterone especially in young healthy men after exclusion of other possible confounding factors. Thus, the aim of our study was to study the possible association of vitamin D status with male hypogonadism among different age groups [12,19].

Aim

The aim of this study was to evaluate the possible relation between vitamin D status and hypogonadism in male patients among different age groups.

Patients and methods

This study was conducted on 80 male patients aged between 20 and 70 years, enrolled from the Endocrinology Unit, Department of Internal Medicine and the Urology Unit, Department of Surgery, Alexandria Main University Hospital. The patients and the controls were divided into two groups. Group I included 40 male patients aged 20–45 years who were further subdivided into two subgroups: group Ia included 20 male patients diagnosed with

hypogonadism and group Ib included 20 eugonadal men serving as a control. Group II included 40 male patients aged 45–70 years and subdivided into two subgroups: group IIa; included 20 male patients diagnosed with hypogonadism and group IIb; included eugonadal men serving as a control.

Diagnosis of hypogonadism in our study was on the basis of biochemical evidence of hypogonadism as the level of total testosterone (TT) is less than 300 ng/dl together with clinical symptoms and signs consistent with hypogonadism.

Patients diagnosed with hypogonadism either primary or secondary were enrolled, where the luteinizing hormone (LH) cutoff level of primary hypogonadism was at least 9.5 μIU/ml and secondary hypogonadism was less than 9.5 μIU/ml. Men of the control group were healthy volunteers with no suggestive features of hypogonadism and normal TT level.

The study was approved by the Ethics Committee (Faculty of Medicine, University of Alexandria), and all study participants provided written informed consent.

Exclusion criteria

Patients with a history of chronic hepatic, renal diseases, diabetes mellitus, history of vitamin D supplementation 6 months before their enrollment or those who received drugs known to affect testosterone levels such as opioids, 5-α reductase inhibitors, antiandrogens and antipsychotics were excluded from the study.

Methods

All enrolled patients were subjected to the following:

(1) Full history with special emphasis on symptoms of hypogonadism including: Reduced or loss of *libido*, morning erection, sleeping pattern, mood changes, physical performance, and history of pathological fractures.
(2) Thorough physical examination stressing upon secondary sexual characters: genital examination, anthropometric measurements including weight, height, BMI, waist circumference (WC), and waist hip ratio [20].
(3) Hormonal assay using enzyme-linked immunofluorescence assay: Serum TT [21], 25 [OH]D_3 [22], LH [23], follicle stimulating hormone (FSH) [23], estradiol (E2) [24], prolactin [24] Kits (ChemuxBioScience Inc., South San Francisco, California, USA.).
    (a) Free androgen index (FAI) was calculated using the equation [100×TT (nmol/l)/ SHBG (nmol/l)].
(4) Assessment of sex-hormone-binding globulin (SHBG) using electrochemiluminescence immunoassay.

Statistical analysis [25]

Data were fed to the computer and analyzed using IBM SPSS software package, version 20.0. (IBM Corp., Armonk, New York, USA) [26]. Qualitative data were described using number and percentage. The Kolmogorov–Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, SD, and median. Significance of the obtained results was judged at the 5% level. The used tests were: (i) χ^2-test: for categorical variables, to compare between different groups. (ii) Fisher's exact or Monte Carlo correction: for correction for χ^2 when more than 20% of the cells have expected count of less than 5. (iii) Student's *t*-test: for normally distributed quantitative variables, to compare between two studied groups. (iv) *F*-test (analysis of variance): for normally distributed quantitative variables, to compare between more than two groups, and post-hoc test (Tukey) for pairwise comparisons. (v) Pearson's coefficient: to correlate between two normally distributed quantitative variables. (vi) Mann–Whitney test: for abnormally distributed quantitative variables, to compare between two studied groups. (vii) Kruskal–Wallis test: for abnormally distributed quantitative variables, to compare between more than two studied groups, and post-hoc (Dunn's multiple comparisons test) for pairwise comparisons.

Results

There was no significant difference between the two subgroups of groups I and II as regards the age [P=0.966, 0.975, respectively], whereas there was a statistically significant difference between patients in groups Ia and IIa as regards the age (P<0.001) (Table 1).

There was a statistically significant difference between the two subgroups of groups I and II as regards TT level being lower in groups Ia and IIa versus groups Ib and IIb (P<0.001 for both). There was no significant difference between patients in groups Ia and IIa as regards TT (P=0.708) (Table 2 and Fig. 1).

There was a statistically significant difference between the two subgroups of groups I and II as regards SHBG level and FAI being lower in hypogonadal men as

Table 1 Distribution of the four studied subgroups according to clinical parameters

	Group I (20–45 years)		Group II (45–70 years)		*F*	*P*
	Cases (*n*=20)	Control (*n*=20)	Cases (*n*=20)	Control (*n*=20)		
Age (years)						
Minimum–maximum	21.0–43.0	22.0–44.0	48.0–68.0	46.0–67.0	96.697*	<0.001*
Mean±SD	32.0±6.03	32.45±6.57	56.90±6.27	56.05±6.60		
Significance between groups	P_1=0.996, P_2=0.975, P_3<0.001*					
Height (cm)						
Minimum–maximum	140.0–177.0	158.0–178.0	146.0–183.0	163.0–182.0	5.935*	0.001*
Mean±SD	163.50±9.04	169.20±6.05	172.25±8.41	172.05±5.93		
Significance between groups	P_1=0.084, P_2=1.000, P_3=0.002*					
Weight (kg)						
Minimum–maximum	51.0–94.0	60.0–105.0	60.0–112.0	62.0–104.0	8.980*	<0.001*
Mean±SD	72.20±11.85	80.95±13.36	91.60±12.82	84.65±9.90		
Significance between groups	P_1=0.108, P_2=0.271, P_3<0.001*					
BMI (kg/m^2)						
Minimum–maximum	22.10–31.50	22.30–35.90	20.0–36.70	18.70–34.20	4.208*	0.008*
Mean±SD	26.90±2.82	28.21±4.02	30.89±3.96	28.68±3.57		
Significance between groups	P_1=0.662, P_2=0.223, P_3=0.004*					
WC (cm)						
Minimum–maximum	77.0–111.0	78.0–108.0	81.0–126.0	81.0–118.0	9.066*	<0.001*
Mean±SD	86.70±8.87	93.35±11.17	103.30±11.98	99.30±10.74		
Significance between groups	P_1=0.214, P_2=0.644, P_3<0.001*					
WHR						
Minimum–maximum	0.92–1.12	0.84–1.12	0.82–1.19	0.95–1.25	0.979	0.407
Mean±SD	1.01±0.06	1.02±0.07	1.03±0.08	1.05±0.06		
Significance between groups	P_1=0.981, P_2=0.745, P_3=0.923					

WC, waist circumference; WHR, waist hip ratio. *F* and *P* values for analysis of variance test; significance between groups was done using post-hoc test (Tukey' test). P_1: *P* value for comparing between cases and control in group I. P_2: *P* value for comparing between cases and control in group II. P_3: *P* value for comparing between cases in groups I and II. *$P \leq 0.05$, statistically significant.

compared with eugonadal men ($P<0.001$ for both). There was no statistical significant difference between patients in groups I and II as regards SHBG level ($P=0.124$) despite being lower in groups Ia versus IIa, whereas there was a statistically significant difference between patients in groups Ia and IIa as regards FAI being higher in group Ia as compared with group IIa ($P=0.021$) (Table 2 and Figs 2 and 3).

Distribution of total cases with hypogonadism in both groups according to luteinizing hormone levels

It was shown that 55% of cases with hypogonadism in both groups Ia and IIa were suffering from primary hypogonadism and 45% were suffering from secondary hypogonadism according to the LH level (cutoff level 9.5 μIU/ml) (Fig. 4).

Vitamin D status in the studied groups

It has been shown that, 73% (29/40) of the hypogonadal patients in both groups and 55% (22/40) of the healthy participants had vitamin D deficiency, whereas 27 (11/40) of the patients and 37.5% (15/40) of the healthy controls had vitamin D insufficiency and only 7.5 % (3/40) of the healthy controls were vitamin D sufficient (Table 3 and Figs 5 and 6).

Comparison between the four studied groups according to 25-hydroxyvitamin D_3 levels

There was no statistically significant difference between the two subgroups of groups I and II as regards serum vitamin D level ($P=0.681$, 0.823, respectively) (Table 4 and Fig. 7). Serum vitamin D level was statistically significantly higher in group IIa as compared with group Ia ($P=0.037$) (Table 4 and Fig. 7).

Correlation between vitamin D and different parameters

In hypogonadal patients (groups Ia and IIa), it was shown that vitamin D was positively correlated with TT, SHBG and FAI in both groups, whereas no significant difference could be detected between vitamin D and other parameters, as shown in Table 5 and Figs 8–10.

Relation between vitamin D levels and total testosterone in men with hypogonadism

It was found that there is a positive correlation between TT and 25[OH]D_3 in hypogonadal men in both groups which is more pronounced in elderly hypogonadal men (Table 6).

Table 2 Distribution of the four studied subgroups according to laboratory data

	Group I (20–45 years)		Group II (45–70 years)		F	*P*
	Cases (*n*=20)	Control (*n*=20)	Cases (*n*=20)	Control (*n*=20)		
Total testosterone (ng/ml)						
Minimum–maximum	110.0–270.0	334.0–742.0	110.0–266.0	348.0–711.0		
Mean±SD	178.75±43.17	494.60±117.28	184.75±41.76	496.35±105.50	59.419*	<0.001*
Significance between groups		P_1<0.001*, P_2<0.001*, P_3=0.708				
Sex-hormone binding globulin (nmol/l)						
Minimum–maximum	19.60–45.20	35.10–77.10	24.50–55.10	57.40–102.0	F=71.098*	<0.001*
Mean±SD	34.66±6.48	55.78±11.38	42.06±8.58	79.64±13.97		
Significance between groups		P_1<0.001*, P_2<0.001*, P_3=0.124				
Free androgen index						
Minimum–maximum	11.76–20.73	24.23–40.0	5.61–16.75	20.39–24.63	H=71.113*	<0.001*
Mean±SD	17.57±2.0	30.50±3.30	14.70±2.34	21.51±1.16		
Significance between groups	17.30	29.95	14.82	21.05		
Minimum–maximum		P_1<0.001*, P_2<0.001*, P_3=0.021*				
Serum estradiol (pg/ml)						
Minimum–maximum	14.40–76.60	21.80–44.80	8.76–44.10	18.60–37.20	H=18.925*	<0.001*
Mean±SD	30.12±13.76	37.59±7.30	26.34±8.15	28.56±6.22		
Median	26.25	40.10	24.60	30.70		
Significance between groups		P_1=0.001*, P_2=0.617, P_3=0.997				
Prolactin (ng/ml)						
Minimum–maximum	3.34–470.0	6.80–21.0	7.0–53.60	7.80–19.50		
Mean±SD	44.61±102.23	10.53±4.20	16.17±11.96	12.96±3.53		
Median	19.70	9.01	13.95	12.30		
Significance between groups		P_1=0.981, P_2=0.745, P_3=0.923				
LH (μIU/ml)						
Minimum–maximum	0.10–30.50	2.60–8.90	0.19–27.30	2.10–9.10	8.540*	0.036*
Mean±SD	10.77±11.83	5.29±1.76	12.70±8.35	6.28±2.04		
Median	8.40	5.32	12.25	7.0		
Significance between groups		P_1=0.252, P_2=0.050*, P_3=0.088				
FSH (μIU/ml)						
Minimum–maximum	0.10–61.80	3.11–10.10	0.51–43.10	4.11–10.70	5.597	0.133
Mean±SD	17.86±22.48	5.48±2.32	17.32±13.09	6.88±2.08		
Median	4.67	4.34	19.90	6.45		
Significance between groups		P_1=1.000, P_2=0.093, P_3=0.310				

FSH, follicle stimulating hormone; LH, luteinizing hormone. *$P \leq 0.05$, statistically significant.

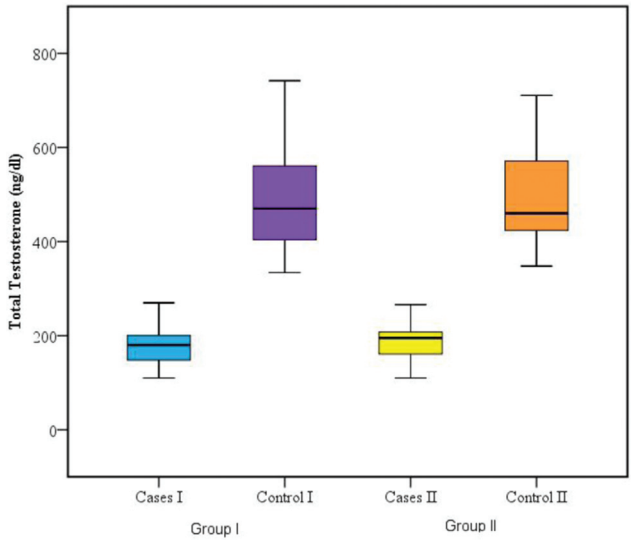
Discussion

Andropause is the gradual reduction of the male sex hormones with increasing age. Associated symptoms are sexual dysfunction, weakness, fatigue, insomnia, loss of motivation, mood disorders, and reduction of bone density [27]. Large cross-sectional studies have concluded that as men age, there is a progressive decline in several sex hormones in particular testosterone (0.5–2% annual decline in TT), dehydroepiandrosterone, and related increase in LH, FSH, and SHBG (around 1% annually) [3,28,29]. It has been appreciated that a progressive decline in TT levels has both testicular and hypothalamopituitary elements [30].

Estrogen and related steroids, thyroid hormone, and insulin increase the SHBG levels. SHBG decreases in response to androgens, in the presence of hypothyroidism and insulin resistance [31]. The cause of the increase in SHBG levels with age remains unclear. Possibly, the age-associated decline in growth hormone and insulin-like growth factor 1 levels might contribute to the increased SHBG levels as it has been observed that growth hormone treatment of adult men with growth hormone deficiency results in a decrease of SHBG levels [29,32].

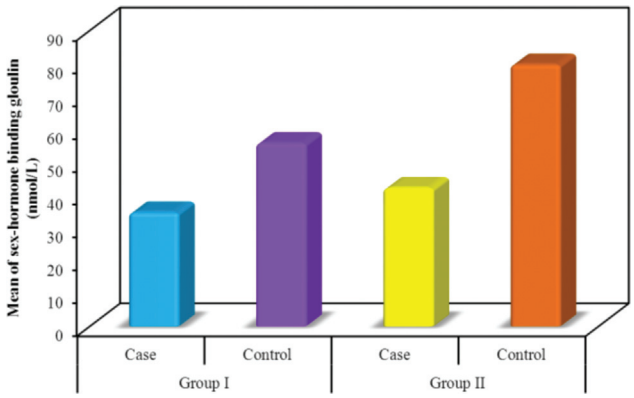
It was found in our study that TT levels and FAI were lower in hypogonadal men (groups Ia and IIa) as compared with control groups (Ib and IIb) in different ages groups, whereas it has been observed that SHBG were lower in control groups as compared with hypogonadal groups, with statistical significant difference between patients and controls in groups I and II as regards TT, FAI, and SHBG. Although, TT levels were not significantly different in hypogonadal men aged 20–45 years (group Ia) as compared with

Figure 1



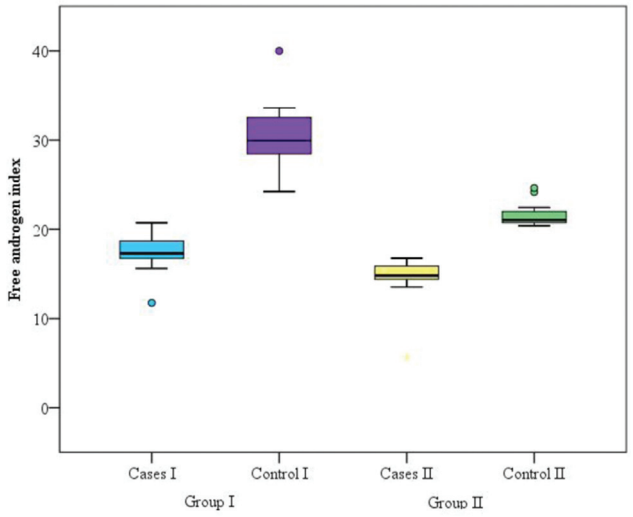
Comparison between the four studied subgroups according to total testosterone. Statistically significant at $P \leq 0.05$.

Figure 2



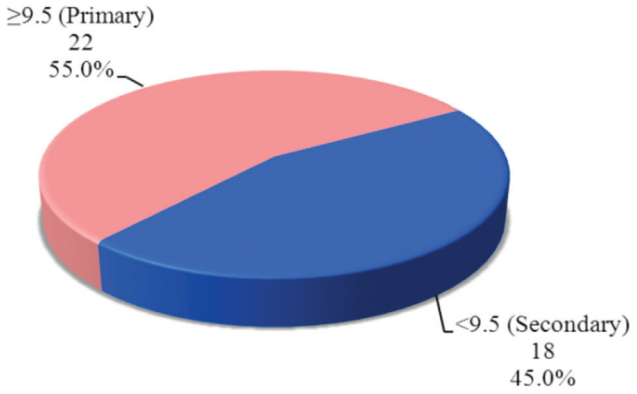
Comparison between the four studied groups according to sex-hormone binding globulin (nmol/l).

Figure 3



Comparison between the four studied groups according to free androgen index.

Figure 4



Distribution of total cases with hypogonadism in both groups according to luteinizing hormone levels:

hypgonadal men aged 45–70 years (group IIa), SHBG was higher in group IIa as compared with group Ia although it did not reach a statistical significance. However, FAI reflecting active testosterone levels was statistically significantly lower in the elderly group (group IIa) consistent with studies supporting decline of male sex steroids.

An interesting study held by Muller *et al.* [29] studied TT, bioavailable testosterone (BT), dehydroepiandrosterone, and SHBG in male patients aged 40–80 years. His group demonstrated a progressive decline in TT, BT, and dehydroepiandrosterone in men with aging 0.2, 0.7, and 1.2%, respectively and a progressive increase in SHBG by 1.1% annually [29]. However, Muller *et al.* [29] sorted the enrolled individuals in their study into those adopting healthy and unhealthy lifestyles. Healthy and/or healthy lifestyle was defined as absence of chronic disease (cardiovascular disease, pulmonary disease, cancer, hypertension, diabetes), chronic use of medication, severe alcohol consumption (>40 g/day), current smoking, and obesity. They found that the general health status modified the effect between TT and age supported by stable TT with age in healthy participants. They concluded that, besides aging, important determinants of sex hormone concentrations were BMI, WC, current smoking, general health status, and physical activity.

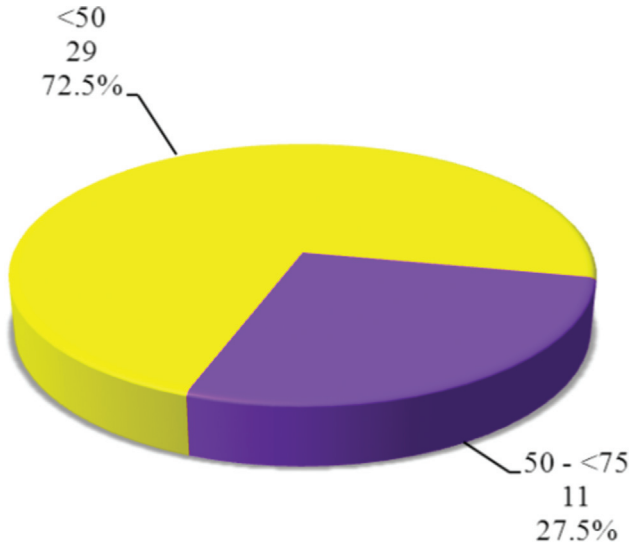
In agreement with our results, Campbell *et al.* [30] studied TT, BT, and SHBG in different age groups from 20 to 90 years among Turkana men into two subpopulations, nomadic and settled pastoralists of

Table 3 Comparison between the two studied groups according to 25-hydroxyvitamin D_3

	Group.1 (cases) (n=40) [n (%)]	Group.2 (control) (n=40) [n (%)]	Test of significance	P
25(OH)D_3 (nmol/l)				
<50	29 (72.5)	22 (55.0)	χ^2=4.171	^{MC}P=0.105
50 to <75	11 (27.5)	15 (37.5)		
≥75	0 (0.0)	3 (7.5)		
Minimum–maximum	31.75–65.0	20.10–80.50	t=1.305	0.196
Mean±SD	42.95±11.42	46.83±14.96		

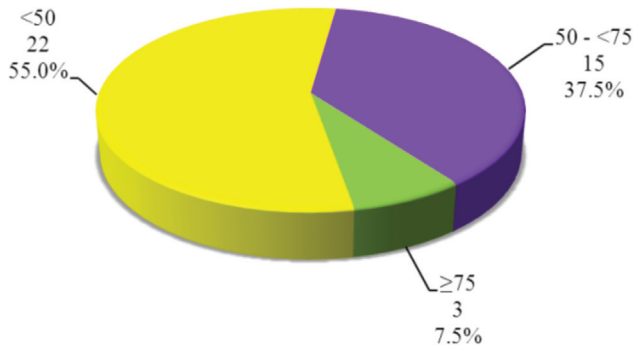
25(OH)D_3, 25-hydroxyvitamin D_3. ^{MC}P: P value for Monte Carlo for χ^2-test for comparing between the two groups; t and P values for Student's t-test for comparing between the two groups.

Figure 5



Distribution of studied sample according to 25-hydroxyvitamin D_3 (nmol/l) in cases group.

Figure 6



Distribution of studied sample according to 25-hydroxyvitamin D_3 (nmol/l) in control group.

Northern Kenya. Campbell *et al.* [30] found that TT did not exhibit a significant linear decline with age in either subgroup, whereas SHBG values showed a significant linear increase among the nomads, and BT showed a significant decrease with age among the nomads.

Most studies on human and animals have shown a positive association between low levels of vitamin D and impaired gonadal function. The authors hypothesize that besides the effect of androgens on serum vitamin D levels; serum vitamin D levels may have a direct impact on gonadal function, with biological plausibility stemming from the presence of VDR in the testis [15], especially in early stages of developing gonads at 16 weeks of gestation [33,34]. Moreover, vitamin D has been implicated in sperm function, maturation, and motility either through a direct effect of vitamin D or indirectly through its effect on calcium homeostasis, where calcium is known to exert a plausible effect on all aspects of sperm function, maturation, and motility [34].

The first study that showed a detailed association between 25-hydroxyvitamin D [25(OH)D] levels with androgens was held in Australia by Wehr *et al.* [17], where the study group aimed to investigate the association of 25(OH)D levels with TT, FAI, and SHBG and whether androgen levels show a similar seasonal variation to 25(OH)D. The study was performed on 2229 men with a mean age of 62±11 years referred for coronary angiography in 2010. They show an independent association of 25(OH)D levels with testosterone, FAI, and SHBG levels after exclusion of any possible confounding factors. The group observed that men with sufficient 25(OH)D concentrations had higher TT, FAI, and SHBG concentrations compared with men with either insufficient or deficient 25(OH)D concentrations. Moreover, there was a seasonal variation of 25(OH)D, testosterone and FAI, with a peak level in August and a nadir in March [17].

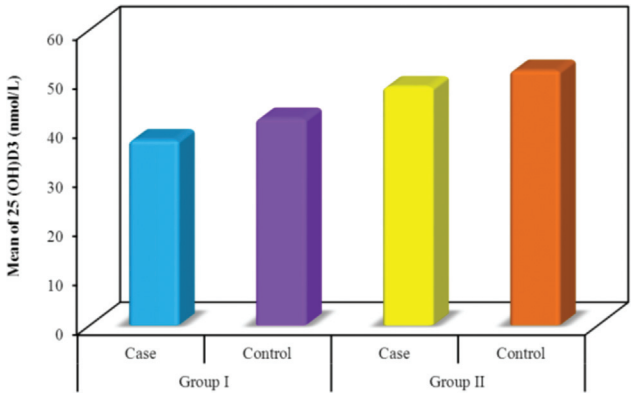
As a trial to demonstrate the effect of vitamin D supplementation on testosterone levels in vitamin D-deficient men, Pilz *et al.* [18], studied the effect of vitamin D supplementation on testosterone levels in 54 men aged 20–49 years. The participants were derived from healthy overweight individuals following weight reduction program. Initial 25(OH)D concentrations were in the deficiency range and testosterone values were at the lower end of the

Table 4 Comparison between the four studied groups according to 25-hydroxyvitamin D_3

25(OH)D_3 (nmol/l)	Group I (20–45 years)		Group II (45–70 years)		*F*	*P*
	Cases (*n*=20)	Control (*n*=20)	Cases (*n*=20)	Control (*n*=20)		
Minimum–maximum	31.75–57.6	20.10–64.0	33.25–65.0	23.5–80.5	5.240*	0.002*
Mean±SD	37.57±7.38	41.94±11.7	48.34±12.3	51.7±16.48		
Significance between groups		P_1=0.681, P_2=0.823, P_3=0.037*				

25(OH)D_3, 25-hydroxyvitamin D_3. *F* and *P* values for analysis of variance test, significance between groups was done using post-hoc test (Tukey' test). P_1: *P* value for comparing between cases and control in group I. P_2: *P* value for comparing between cases and control in group II. P_3: *P* value for comparing between cases in groups I and II. *$P \leq 0.05$, statistically significant.

Figure 7



Comparison between the four studied groups according to 25-hydroxyvitamin D_3.

reference range. The participants were offered either a daily supplementation of 83 μg (3332 IU) vitamin D or a placebo as part of a double-blind randomized controlled trial. Their results suggested that vitamin D supplementation may increase testosterone levels. However, owing to the small sample size and as men were participating in a diet program for weight reduction, the validity of the results was questionable [18].

Lee *et al.* [35] from the European male aging study studied the association of hypogonadism with vitamin D status in a cross-sectional survey of 3369 community-dwelling men aged 40–79 years in eight European centers. Results have shown that among generally healthy, community-dwelling men, low vitamin D levels, as assessed by serum 25(OH)D (<50 nmol/l), are significantly associated with biochemical hypogonadism based on combined TT and LH measurements. However, these positive association between 25(OH)D and free testosterone is abrogated when adjusted for age, BMI, smoking, alcohol, physical activity, hypertension, diabetes, and depression. It also showed no seasonal variation of testosterone in contrast to the study by Wehr *et al.* [17].

Another study in 2012 held by Nimptsch *et al.* [36], who investigated the cross-sectional association of plasma 25(OH)D levels and TT and free testosterone measured by immunoassay in 1362 male participants of the health professional follow-up study who were selected for nested case–control study on prostate cancer. Results have shown that 25(OH)D was positively associated with TT and free testosterone levels but they did not observe parallel seasonal variation patterns which support results previously reported by Lee *et al.* [35].

In our study, in accordance with the previous studies, our results showed a significant association between low 25(OH)D levels and hypogonadism in men. Vitamin D was positively correlated with TT and FAI, and SHBG, whereas no significant difference could be detected between vitamin D and E2, prolactin.

It has been estimated that 20–100% of the USA, Canadian, and European elderly men and women are vitamin D deficient [37]. Children and young and middle-aged adults are at equally high risk for vitamin D deficiency and insufficiency worldwide [37,38]. Interestingly, our results showed that vitamin D deficiency and insufficiency are highly prevalent in our studied cohort in both hypogonadal and eugonadal men. In our study, we found that 73% (29/40) of the hypogonadal men in both groups and 55% (22/40) of eugonadal men had vitamin D deficiency, whereas 27% (11/40) of the hypogonadal and 37.5% (15/40) of eugonadal men had vitamin D insufficiency and only 7.5 % (3/40) of the eugonadal men were vitamin D sufficient. Wehr *et al.* [17] also reported that 63% of the participants have vitamin D deficiency of less than 50 nmol/l, 25.6% have vitamin D insufficiency (50 to <75 nmol/l), and 11.4% have vitamin D sufficiency, whereas our results showed no hypogonadal men with vitamin D sufficiency.

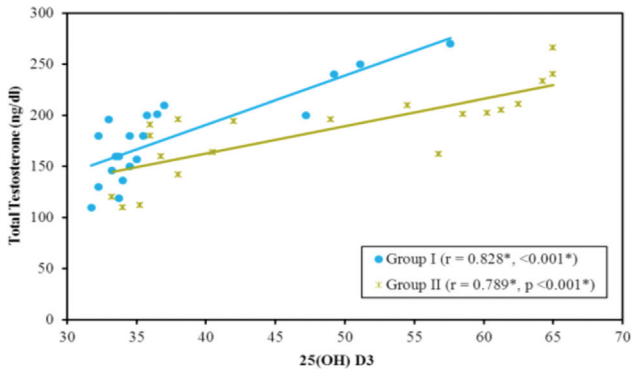
Although in our study, a significant positive association was elicited between 25(OH)D and TT, FAI, and SHBG among hypogonadal men in both age groups in agreement with the previous mentioned studies, we failed to find a statistically significant difference

Table 5 Correlation between 25-hydroxyvitamin D_3 with different parameters

	Cases (*n*=40)			
	Group I (20–45 years)		Group II (45–70 years)	
	r	*P*	*r*	*P*
Total testosterone (ng/dl)	0.828*	<0.001*	0.789*	<0.001*
Sex-hormone binding globulin	0.685*	<0.001*	0.446*	0.049*
Free androgen index	0.723*	<0.001*	0.579*	0.008*
Serum estradiol (pg/ml)	–0.254	0.279	–0.027	0.909
Prolactin (ng/ml)	–0.041	0.862	–0.284	0.226

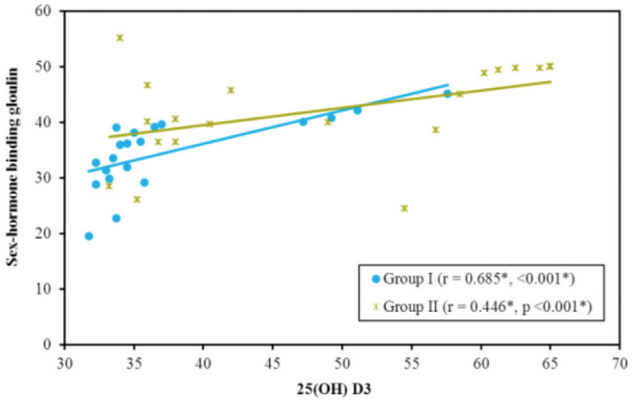
r, Pearson's coefficient. *$P \leq 0.05$, statistically significant

Figure 8



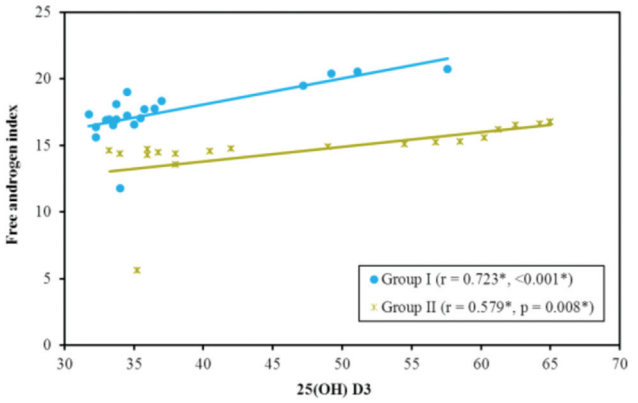
Correlation between 25-hydroxyvitamin D_3 [25(OH)D_3] with total testosterone (ng/dl).

Figure 9



Correlation between 25-hydroxyvitamin D_3 [25(OH)D_3] with sex-hormone binding globulin.

Figure 10



Correlation between 25-hydroxyvitamin D_3 [25(OH)D_3] with free androgen index.

between vitamin D levels among hypogonadal men and their age-matched eugonadal controls in both groups I and II. This may be attributed to the high prevalence of vitamin D deficiency in all studied cohort. Furthermore, the small sample size may serve as another explanation.

Most studies have shown higher prevalence of vitamin D deficiency in the elderly [39,40]. In contrast, in a study in Tehran assessing the prevalence of vitamin D in elderly deficiency, the study group observed that elderly women demonstrated statistically significant higher serum levels of vitamin D compared with the young and middle-aged women. They proposed that parenteral vitamin D intake by the elderly was the major differentiating factor between various age groups that could explain the high prevalence of a higher levels of vitamin D in the elderly women. It was suggested that vitamin D is frequently prescribed to elderly women due to multiple musculoskeletal complaints given the long half-life of vitamin D, even in those who were receiving vitamin D in the preceding months, vitamin D will show relatively higher levels [39].

Similarly, mean serum 25(OH)D was found to be significantly statistically higher in the elderly hypogonadal men in our study as compared with younger group. Consistent with our results, Wang *et al.* [41], conducted a cross-sectional study of 2854 men with a mean age of 53±13 years aiming to investigate the association between vitamin D levels and testosterone, SHBG, E2 and hypogonadism in Chinese men. Results of their study have shown that a lower vitamin D level was associated with a higher prevalence of hypogonadism in Chinese men. They observed that lower 25(OH)D levels were significantly

Table 6 Relation between 25-hydroxyvitamin D_3 and total testosterone (ng/dl) in hypogonadal men (groups Ia and IIa)

	20–45 years (*n*=20)		45–70 years (*n*=20)	
	25(OH)D_3		25(OH)D_3	
	<50 (*n*=18)	50 to <75 (*n*=2)	<50 (*n*=11)	50 to <75 (*n*=9)
Total testosterone (ng/dl)				
Minimum–maximum	110.0–240.0	250.0–270.0	110.0–196.0	162.0–266.0
Mean±SD	169.72±34.76	260.0±14.14	160.45±34.42	214.44±29.29
Z (*P*)	2.273* (0.023*)	3.306 (0.001*)		

25(OH)D_3, 25-hydroxyvitamin D_3. *Z* and *P* values for Mann–Whitney test for comparing between the two groups. *$P \leq 0.05$, statistically significant.

associated with lower TT, E2, SHBG, LH, and FSH levels after adjusting for age, residence area, economic status, and current smoker. Moreover, Wang *et al.* [41] found higher serum vitamin D levels in elderly men as compared with younger age groups and they tried to explain their finding as they thought that young individuals experience more indoor and sedentary activities, whereas the older, retired men may engage in more outdoor activities that result in more sun exposure.

In contrast to our findings and to most of the results yielded from the previous studies showing a positive linear association between vitamin D status and testosterone levels in men, other studies support an inverse U-shaped association between the two variables [12,19]. They observed that men with both low and high 25(OH)D concentrations demonstrate poorer gonadal function compared with those with intermediate vitamin D levels

Hammoud *et al.* [19] reported a U-shaped association between 25(OH)D concentration and semen quality and hormonal parameters including testosterone in a cross-sectional study of 152 healthy men. Men with higher (≥50 ng/ml) and lower (<20 ng/ml) 25(OH)D levels presented worse sperm concentration and motility compared with men with vitamin D between 20 and 50 ng/ml).

Similar to the previous study, Lerchbaum *et al.* [12] reported in a cross-sectional study on 225 men, a U-shaped association between vitamin D and hypogonadism, as they observed that, men within the highest (>102 nmol/l) and lowest (≤43.9 nmol/l) 25(OH)D quintiles had an increased risk of hypogonadism, whereas hypogonadism risk was lowest in men with 25(OH)D levels of between 82 and 102 nmol/l.

As regards this particular U-shaped association between 25(OH)D and gonadal function, the increased risk of hypogonadism in men with higher vitamin D concentration is difficult to interpret. It has been hypothesized that high vitamin D levels may affect vitamin D metabolism within the target tissues, leading to increased 24-hydroxylation [42]. Thus, in the presence of high circulating 25(OH)D levels, the concentration of the biologically active 1,25 (OH)D_3 might be reduced in target tissues such as testis and the pituitary gland. The findings proposed by Lerchbaum and colleagues [12,19] must be interpreted cautiously as proposed by some researches as their findings point to a possible deleterious effects of vitamin D supplementation on gonadal function when the levels of vitamin D exceeded their suggested cutoff limits.

The association between obesity and hypogonadism is bidirectional and both conditions are mutually interrelated [43]. Obesity-related male hypogonadism is now considered as a form of hypogonadotropic hypogonadism [44]. Data from the European Male Aging Study show that, obesity was associated with an 8.7-fold and overweight with a 3.3-fold increased relative risk of secondary hypogonadism [35]. In contrast, low testosterone levels is associated with increased visceral fat mass and adipocyte dysfunction [43]. Furthermore, previous studies have reported that obesity is associated with low serum 25(OH)D levels [45]. It has been found that VDR is expressed in human adipocytes and that vitamin D deficiency leads to up regulation of parathyroid hormone, which in turn increases free intracellular calcium in adipocytes, which blunts the lipolytic response to catecholamines and enhances lipogenesis [46].Our results showed a statistically significant difference between young hypogonadal patients and older patients as regards BMI and WC being higher in elderly age group as compared with the younger group. However, no significant difference could be found between hypogonadal patients compared with eugonadal controls. In contrast to our findings, Lerchbaum *et al.* [12] reported significant differences between eugonadal and hypogonadal men as regards BMI,

WC, and waist hip ratio. Similarly, Wang *et al.* [41] reported a significant difference in BMI between men with hypogonadism and controls. Failure to show a significant difference as regards markers of obesity between hypogonadal and eugonadal men may be partly explained by the small sample size, on one hand and to the high prevalence of vitamin D deficiency in all the four studied subgroups on the other hand.

It has been well appreciated that E2, plays an important role in men, the most recognized role is its effect on the bone mass [47]. The majority of circulating E2 in men is primarily derived from the peripheral aromatization of circulating testosterone by adipocytes located throughout the body into estrogen [48]. Furthermore E2 can be also synthesized in certain sites, where E2 is required for its normal tissue homeostasis such as the bone, brain, and the hypothalamus [49]. In our study, we found that E2 was significantly lower in hypogonadal patients compared with eugonadal controls in both groups, although it did not reach a statistically significant difference in the older group (group II). Wang *et al.* [41] showed significantly lower E2 levels in hypogonadal patients which were similar to our results; however Lerchbaum *et al.* [12], in their study failed to show significant difference in E2 on comparing hypogonadal patients with eugonadal men.

The majority of studies support a positive, linear association between 25(OH)D concentrations and testosterone concentrations, whereas some researchers have highlighted the possible negative effects of high 25(OH)D levels. This apparent conflicting data may be attributed to the differences in study design, baseline 25(OH)D concentration, different proportion of men with vitamin D sufficiency, dietary vitamin D intake, age and ethnicity among the participants as well as assay methodology. Further interventional studies are needed to prove a causal relationship and whether vitamin D exerts its effects on reproductive male function directly or indirectly through other vitamin D regulated endocrine factors, such as calcium or estrogen levels, that may play an important role in reproductive outcomes. Finally, it is recommended to study the beneficial effects of vitamin D supplementation in a large cohort of hypogonadal men (specially classified into primary or secondary) in terms of normalization of testosterone levels aiming to enhance their sense of well-being and their quality of lives.

Conclusion

Low vitamin D is associated with male hypogonadism across all age groups, this association is more pronounced in elderly hypogonadal men.

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Conflicts of interest

There are no conflicts of interest.

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