Association Between Vitamin D Levels and Semen Parameters in Infertile Males

İnfertil Erkeklerde D Vitamin ve Semen Parametreleri Arasındaki İlişki

D Vitamin ve Semen Parametreleri / Vitamin D and Semen Parameters

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Özet

Amaç: Bu çalışmanın amacı, yıkama sonrası ileri hareketli sperm sayısına göre infertil erkeklerin klinik ve laboratuvar özelliklerini karşılaştırmak ve serum D vitamini (VD) düzeyleri ve semen parametreleri arasında bir ilişki olup olmadığını değerlendirmektir. Gereç ve Yöntem: Toplam 198 infertil erkek bu kesitsel çalışmaya dahil edildi. Çalışma popülasyonu temel olarak yıkama sonrası toplam ileri hareketli sperm sayısına (TİHSS) göre 5 milyon / ml'den az (çalışma grubu) ve 5 milyon / ml veya daha fazla (kontrol grubu) olarak iki gruba ayrıldı. Her bir hasta için kaydedilen ana parametreler; yaş, VKİ (vücut kitle indeksi), infertilite tipi, infertilite süresi, önceki ameliyat, hastalık, sigara, ilaç kullanımı ve serum 25OHVD3, toplam kalsiyum (Ka) ve testosteron (TT) düzeyleri, gonadotropinler, ve semen parametreleriydi. Bulgular: Gruplar arasında yaş, VKİ, infertilite tipi, infertilite süresi, önceki operasyon, hastalık, sigara, ilaç kullanımı, TT ve 25OHVD3 açısından istatistiksel olarak anlamlı farklılık yoktu. Serum gonadotropin düzeyleri çalışma grubunda anlamlı olarak düşük bulundu (p <0.001). Tüm grupta ortalama 25OHVD3 düzeyi 21,0 ± 7,2 ng / ml idi ve çalışma grubunda VD ile yıkama öncesi / sonrası toplam sperm sayısı arasında pozitif bir korelasyon vardı (sırasıyla, p = 0,036, p = 0,034). Serum Ka seviyesi yine bu grupta anlamlı olarak daha düşüktü (p = 0,012). Tartışma: D vitamini yetersizliği infertil erkeklerde yaygındı. VD ve Ka desteği yıkama sonrası TİHSS 5 milyondan / ml'den az, VD ve Ka eksikliği gösterilmiş infertil erkekler için uygun tedavi olabilir. Bu konuda iyi tasarlanmış ve büyük serili ileri prospektif kontrollü çalışmalara ihtiyaç vardır.

Anahtar Kelimeler

Kalsiyum; Erkek İnfertilitesi; Semen Parametreleri; D Vitamini

Abstract

Aim: The purpose of this study was to compare clinical and laboratory characteristics of infertile males according to their postwash progressively motile sperm count and to evaluate whether there was a relationship between serum vitamin D (VD) levels and semen parameters. Material and Method: A total of 198 infertile men were included in this cross-sectional study. Study population was mainly divided into two groups according to post wash total progressively motile sperm count (TPMSC) as less than 5 million/ml (study group) and equal or greater than 5 million/ml (control group). The main parameters recorded for each patient were; age, BMI (body mass index), infertility type, infertility duration, previous operation, history of disease, smoking, drug usage and serum levels of 25OHVD3, total calcium (Ca) and testosterone (TT), gonadotropins, and semen parameters. Results: There were no statistically significant differences between groups in terms of age, BMI, infertility type, infertility duration, and previous operation, history of disease, smoking, drug usage, TT, and 25OHVD3 levels. Serum levels of gonadotropins were significantly lower in the study group (p<0.001). The mean 25OHVD3 level was 21.0±7.2 ng/ml in the total group and there was a positive correlation between VD and pre/post wash sperm count in the study group (p=0.036, p=0.034, respectively). Serum Ca level was also significantly lower in this group (p=0.012). Discussion: Vitamin D insufficiency was common among the infertile men. VD and Ca supplementation may be appropriate treatment for infertile males with documented VD and Ca deficiency, whose post wash TPMSC lesser than 5 million/mL. Further well designed and large series prospective controlled studies are needed on this issue.

Keywords

Calcium; Male Infertility; Semen Parameters; Vitamin D

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Introduction

Infertility is defined as the failure to conceive after one year of unprotected intercourse with the same partner [1]. Male factor is one of the most frequent causes of infertility (40-50%) [2]. Many infertile men are experiencing low total sperm count or different semen abnormalities such as low sperm motility and impaired sperm function, thus resulting in inability to fertilize an oocyte in the absence of a specific underlying etiologic factor [3]. Different cellular abnormalities were defined which reduce semen parameters at molecular and biochemical levels [4]. VD is primarily involved in metabolism of calcium-phosphorus and regulates bone mineralization. It is a fat soluble vitamin and provides intestinal absorption of these minerals, and also is regarded as a steroid hormone. The main source is sunlight induced synthesis in the skin. Cholesterol is converted into cholecalciferol (inactive VD3) by ultraviolet-B rays in this processes and it is hyroxylated by hepatic 25-hydroxylase subsequently and renal 1alpha-hydroxylase to form 1,25(OH)2VD3 (active VD3-calcitriol). A small amount of circulating VD is supplied by diet or food supplements.

Recent human and animal studies have shown that VD is important for both male and female reproductive functions, and VD receptor and metabolizing enzymes are expressed in male genital tract and germ cells [4, 5]. Expression of a VD inactivating enzyme in spermatozoa distinguishes normal and infertile men with a high specificity and suggested as a marker for both semen quality and VD responsiveness [6].

There is no consensus on definitive reference value for an optimal serum VD levels, yet. However, VD deficiency is considered as a 25OHVitD3 below 20 ng/ml and VD insufficiency as a 25OHVitD3 of 21–29 ng/ml. When the serum levels of VD is higher than 30 ng/mL generally accepted as sufficient [7]. In this study we aimed to evaluate serum VD levels of infertile males and to determine whether there is an association with semen parameters.

Material and Method

This cross-sectional observational study was conducted at the Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey, which is a referral medical center located in the middle region of Turkey. A total of 198 infertile men were recruited from infertility and andrology outpatient clinics between September 2013 and November 2013, after receiving approval of the hospital's local ethics committee. Informed consent was obtained from the each participant. The study was carried out during autumn time in Ankara (Longitude: 40° 4' N, Latitude: 32° 34' E, Altitude: 891 m, the average temperature is 7-18.7 °C). The weather was rainy about 6 days of each month during the study period. Also 6 hours per day was sunny during the study period.

Semen sample and blood sample were taken on the same day. The exclusion criteria are anabolic steroid intake, some supplementation of VD, obstructive azoospermia and other causes of infertility except for male factor and unexplained infertility. Whenever the semen parameters were compatible with azoospermia or oligozoospermia, it was accepted as male factor infertility. Unexplained infertility was defined after excluding common causes of infertility using standard fertility investigations, which include semen analysis, assessment of ovulation, and tubal patency test. Men whose wives had previously no pregnancy were regarded as primary infertile; if pregnancy occurs at least once it was considered as secondary infertility. The data obtained from patients were age, BMI (body mass index), infertility type, and duration of infertility, previous operation, history of disease, smoking, drug usage and obstetrical history of their partners. The cohort was mainly divided into study and control groups according to postwash total progressively motile sperm count (TPMSC) that a value of less than 5 million/ml (n: 93) constituted the study group and a value of greater than 5 million/ml (n: 105) constituted the control group.

All of the patients were asked to respect a period of sexual abstinence for 3-5 days. Semen samples were obtained from the patients by masturbation in a private room nearby the laboratory. After liquefaction for 30 minutes at room temperature, the collected semen specimens (pre-washed) were assessed for conventional semen parameters including sperm concentration and sperm motility by the computer-assisted semen analyzer. The rest of the semen was processed using standard swim up method with a sperm preparation media (Ferticult Flushing mediumTM, FertiProNV, Beernem, Belgium). Post wash analysis was again performed by the computer-assisted semen analyzer. Sperm analysis was performed by the same andrology laboratory technician according to a quality control program.

Blood samples were taken after a minimum of 8 hours fasting. Ten mL of venous blood was drawn from each participant and they were evaluated for some hormonal and biochemical parameters including total testosterone (TT), luteinizing hormone (LH), follicle stimulating hormone (FSH), calcium (Ca) and 25OHVitD3. Serum levels of 25OHVitD3 were measured by using an ELISA Kit; Immunodiagnostic AG, Germany. All blood samples were analyzed at laboratory of hormone and biochemistry of our hospital. The intra-assay and inter-assay coefficients of variation were 8.9% and 10.6% for serum 25OHVitD3.

Statistical analysis

Statistical Package for the Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Statistical significance was set at $p<0.05$. Means and standard deviations for quantitative data and numbers and percents for qualitative data were computed. Independent-samples t test was used to compare unadjusted means of VD between groups. Non parametric variables between groups were compared through Mann-Whitney U test. Pearson correlation test was used for assessing the associations between VD and other parameters.

Results

The mean age of patients was 30.8±5.4 years, mean 25OHVitD3 level was 21.0±7.2 ng/mL and mean BMI was 25.8±3.6 kg/m2 in this study. The men in this sample was mainly divided into two groups, the study (n:93) and the control (n:105) group, according to postwash TPMSC as<5 million/ml and ≥5 million/ml. There was no statistically significant difference between two groups in terms of age, BMI, VD, TT and infertility duration ($p>0.05$). However, there was a statistically significant difference according to FSH, LH and semen parameters ($p<0.001$). And Ca levels were statistically significantly lower in the study group ($p<0.05$). The clinical characteristics and laboratory pa-

Table 1. The clinical characteristics and laboratory parameters of the infertile males and comparison of study and control groups.

	Total group (n:198)	Study Group (<5 m/mL) (n: 93)	Control Group (≥5m/mL) (n: 105)	P value
Age (years)	30.8±5.4	30.8±5.3	30.8±5.5	0.935
Infertility duration (years)	4.4±4.0	4.7±3.8	4.1±4.1	0.298
Semen volume (cc)	2.5±1.4	2.2±1.5	2.8±1.2	0.002
Total sperm count (million)	101.3±126.1	20.5±25.1	172.8±136.2	<0.001
Sperm concentration (million/mL)	36.1±38.8	7.4±8.7	61.6±37.4	<0.001
Progressively motility (%)	22.5±18.4	5.0±7.3	38.0±8.9	<0.001
Non-progressive motile (%)	10.2±7.3	8.2±8.8	11.9±5.3	<0.001
Kruger (%)	4.4±3.5	1.5±1.4	6.9±2.7	<0.001
FSH (IU/mL)	6.8±8.7	10.2±11.5	3.7±2.2	<0.001
LH (IU/mL)	5.5±5.2	7.3±6.9	3.8±1.9	<0.001
TT (ng/dL)	444.2±170.5	446.1±194.9	442.6±146.3	0.884
Total Ca (mg/mL)	9.7±0.5	9.6±0.5	9.8±0.4	0.012
BMI (kg/m2)	25.8±3.6	26.3±4.0	25.4±3.2	0.096
Vitamin D (ng/mL)	21.0±7.2	21.4±7.5	20.7±6.9	0.464

FSH: Follicle stimulating hormone, LH: Luteinizing hormone, TT: Total testosterone, BMI: Body mass index, Ca: calcium. Data are presented as mean±standard deviation. P<0.05 is considered statistically significant.

rameters of study population have been summarized in Table1. The half of the patients was current smokers. Primary infertility (89.9%) is more common among the patients. The mean 25OHVitD3 level was significantly lower in secondary infertile males (p:0.022) (Table2). Correlation analysis showed that there was a negative correlation between VD levels and BMI (r:-0.172; p:0.016), a significant negative correlation between TT levels and BMI (r:-0.332;p<0.001) and a positive correlation between VD and TT (r:164, p:0.021) in the total group. There was no statistically significant correlation between VD and serum Ca levels (p>0.05). There was a negative correlation between VD and age (p:0.002), and there was a positive correlation between VD and pre and postwash sperm concentration in the study group (p<0.05) (Table 3).

Table 2. Comparison of Vitamin D levels according to different variables in the total group.

	n (%)	Vitamin D levels (Mean±SD)	p
Infertility type			
Primary	178 (89.9)	21.4±7.2	0.022
Secondary	20 (10.1)	17.5±6.1	
Liquefaction			
Normal	178 (89.9)	20.8±7.1	0.283
Abnormal	20 (10.1)	22.7±7.6	
Leucospemia			
(+)	28 (14.1)	21.7±6.5	0.587
(-)	170 (85.9)	20.9±7.3	
Smoker	99 (50)	21.0±6.7	0.901
Non smoker	99 (50)	21.1±7.7	

SD:standard deviation. P<0.05 is considered statistically significant.

Table 3. Correlation analysis between Vitamin D levels and other clinical and laboratory parameters.

	Total group (n:198)		Study Group (<5 m/mL) (n: 93)		Control Group (≥5m/mL) (n: 105)	
	Vitamin D					
	r	p	r	p	r	p
Age	-0.137	0.054	-0.321	0.002	0.0297	0.764
Spouse age	-0.128	0.072	-0.304	0.003	0.0134	0.892
Infertility duration	-0.165	0.020	-0.231	0.026	-0.116	0.239
Infertility type	-0.163	0.022	-0.195	0.061	-0.135	0.169
Testosterone	0.164	0.021	0.167	0.110	0.162	0.099
Calcium	-0.041	0.564	-0.116	0.269	0.062	0.532
Semen volume	0.011	0.877	0.0342	0.745	0.010	0.919
Total sperm count	-0.061	0.392	0.217	0.036	-0.099	0.316
Postwash sperm count	-0.074	0.299	0.221	0.034	-0.117	0.236
TPMSC	-0.086	0.226	0.136	0.194	-0.118	0.232
BMI	-0.172	0.016	-0.204	0.050	-0.151	0.124

r: correlation coefficient, P<0.05 is considered statistically significant.

Discussion

The main finding of this cross sectional study was that VD positively correlated with pre and postwash sperm concentration in infertile men whose postwash TPMSC lesser than 5 million/mL. And also serum Ca levels were statistically significantly lower in this group of men than those whose postwash TPMSC equal to or greater than 5 million/mL. In the present study we divided the cohort into two groups according to the postwash progressively motile sperm count as<5 million/ml and ≥5 million/ml. Because, Van Weert et al. [8] designed a meta-analysis to assess the performance and clinical value of the postwash total motile sperm count to predict intrauterine insemination outcome. According to this study, a cut off value of 5 million/mL of the postwash motile sperm count achieved the highest sensitivity and specificity for pregnancy rate.

In a population based observational study mean 25OHVitD3 level was found 37.4±14.0 ng/mL in white men who were selected from general population [9]. Studies in western countries have shown that blood VD levels vary by season and that the prevalence of VD deficiency was higher during winter [10]. Nanri et al. [11] observed a high prevalence of VD deficiency in a Japanese working population in late autumn. There are only few studies conducted on the VD status of adults in Turkey. In a study carried out in the noncoastal areas of the Aegean region during winter time, it was reported that the mean 25OHVitD3 was 20.70±15.50 ng/mL in male participants [12]. Our study season was consistent with mid autumn season; the second season was seen as the lowest levels of VD.

VD levels were significantly lower in secondary infertile males than primary infertile males in our study. There is no data about infertility type and VD relationship in the literature. However, VD level was significantly negatively correlated with age in the study group. This relationship between infertility type and VD may be due to this correlation. Because, the mean age of men with primary infertile was lower than those with secondary infertile. Smoking has a negative impact on semen parameters [13]. Although there was no relationship between smoking and

VD, smoking was very common (%50) in this cohort. Leukospermia is physiologic and improves semen quality in lesser than 10^6 cells/mL, but above 10^6 cells/mL commonly indicates a prostatic infection. Rodin et al. found that leukospermia did not correlate with semen parameters and it was a poor marker for impaired semen quality [14]. In this study, we did not find an association between vitamin D levels and leukocytospermia. It was suggested that there might be a significant relationship between VD and human fertility [15]. In different animal and human studies, VD receptor and VD metabolizing enzymes were shown in male genital tract and germ cells at different stage of spermatogenesis [6, 16]. These studies make us think that VD has an important role in spermatogenesis. In addition, a group of researchers revealed that VD deficiency reduced fertility rates of male rats [17] and this effect could be compensated by Ca replacement lonely [18]. We found that serum total Ca levels were statistically significantly lower in the study group than the control group. The mean VD levels were also lower than the defined reference levels. This result is compatible with the literature when we looked from the perspective of VD's role in reproductive functions.

Bloomberg and colleagues showed that there was a positive correlation between VD levels and sperm morphology and motility [19]. They suggested that VD receptor activation via VD increased intracellular calcium by releasing Ca from intracellular Ca storage in the neck of spermatozoa and induced sperm motility and acrosome reaction in an in vitro study. In another study found out that there was an association between higher VD levels and lower median total sperm count and percentage of normal sperm morphology [20]. But this tendency got lost in the multivariate model adjusting for confounding factors. And they concluded that low VD is not a risk factor for poor semen quality in young healthy men. In our research the total study group consisted of only infertile males and they frequently had poor semen parameters due to male factor. We found that this group of men had VD insufficiency. Although we stratified them according to different semen parameters, statistically significant results were not found between these groups. We only detected a positive correlation between VD and sperm concentration in the total group.

Hammoud et al. [21] showed that serum 25OHVitD3 levels at high (>50ng/mL) and low (<20ng/mL) levels can be negatively associated with semen parameters. In addition they found a negative association between serum VD levels and BMI in their study. We also found a negative correlation between VD and BMI. Their study population was selected from general population and there is no data about their history of fertility. But we can conclude that VD levels are low and sperm parameters are poor in all infertile males according to our study.

In a population based study of middle aged and older men VD was found positively correlated with total and free T [22]. As in their study, we also found that VD was positively correlated with TT. Svartberg et al. [23] reported a bimodal seasonal variation in TT levels and a significant peak between October and November and a rare in June. In different studies considering regulatory role of VD on calcium metabolism, frequently bone mineral densitometry was also studied with sperm parameters. Yang et al. [24] suggested that infertile men have lower testosterone and bone mineral densitometry (BMD) than fertile men. VD and testosterone were both associated with low BMD and low sperm quality in infertile men. VD has been studied in different patient groups and different communities' until now. Therefore different levels of VD were found in those studies. In this study, we found that VD insufficiency was common among the infertile males.

This study focuses on the relationship between vitamin D and sperm parameters is one of the first studies. The strengths of our study are the number of patients and high quality standards of the andrology laboratory. The limitations are its cross-sectional design, the lack of control group consisting of fertile men, and the lack of knowledge of the ethnicity and socioeconomic status of the patients. Another limitation of the current study is that kits for 25OHVitD3 have large variation.

In conclusion, although we cannot rule out whether VD insufficiency is highly prevalent in the general Turkish population, VD insufficiency was common among the infertile males. VD and Ca supplementation may be appropriate treatment for azoospermic and severe oligoastenoteratospermic infertile males with documented VD and Ca deficiency. Further well designed and large series prospective controlled studies are needed on this issue.

Competing interests

The authors declare that they have no competing interests.

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