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Original Research

Is serum vitamin D level a risk factor for idiopathic male fertility?

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Abstract

Background: Idiopathic male infertility is a health problem that is increasingly common worldwide. Aetiology of idiopathic male infertility is still controversial. In this cross-sectional retrospective study, we aimed to investigate the relationship between serum vitamin D level and sperm quality in patients with idiopathic male infertility. **Methods**: Between June 2018 and June 2020, 297 patients including 147 men with idiopathic infertility (as a study group) and 150 fertile men (as a control group) were retrospectively enrolled into the study. Blood samples were collected, and these samples included serum sex steroids, serum vitamin D levels, glucose tests, lipid profiles, liver function tests and kidney function tests. At least two sperm analyses, scrotal doppler ultrasonography and karyotype analysis were performed on each of the patients. Demographic, laboratory and radiological features were also recorded. The Mann Whitney-U test was used to compare groups and quantitative independent data. The Chi-square test was used for qualitative independent data. Spearman's correlation analysis was applied for correlation. Significant results were investigated and analysed further using the logistic regression test. **Results**: The mean age of the patients was 31.98 $\pm$ 6.97 years. The mean serum vitamin D level of the patients was 23.16 $\pm$ 10.40 ng/dL and the mean infertility duration of patients with idiopathic infertility was 29.88 $\pm$ 28.86 months. We observed statistical significance in terms of serum vitamin D levels, impaired total sperm motility, progressive sperm motility and sperm morphology in idiopathic infertile men when compared to fertile men. There were no statistically significant between idiopathic infertile men and fertile men in terms of serum testosterone levels. **Conclusions**: We observed a positive correlation between serum vitamin D levels and impaired sperm parameters, specifically in terms of sperm morphology, total sperm motility and progressive sperm motility. Vitamin D supplementation may be a beneficial contribution to achieving high paternity rates in men with idiopathic male infertility.

Keywords: Infertility; Vitamin D; Testosterone; Semen; Spermiogram

1. Introduction

Idiopathic male infertility has grown to be one of the most common health problem in the world, affecting more than 70 million people per year worldwide [1]. Infertility is defined as the inability of a sexually active, non-contraceptive couple to achieve spontaneous pregnancy within one year. Approximately 15% of couples fail to achieve pregnancy within one year and apply to a specialist for infertility-related medical treatment [2]. A factor of male infertility is found in 50% of involuntarily childless couples, often accompanied by abnormal semen parameters. In 30–40% of these cases, no male-related factors were found to explain the deterioration of sperm parameters. This was historically referred to as idiopathic male infertility. Unexplained male infertility is defined as infertility of unknown origin with normal sperm parameters and partner evaluation. Unexplained infertility rates are assumed to be between approximately 20–30%. Recently, it is considered that idiopathic male infertility may be associated with unidentified pathological factors, including endocrine disruption, environmental pollution, generation of reactive oxygen species (ROS)/sperm DNA damage or genetic and epigenetic abnormalities [3].

Vitamin D (25(OH)D) is liposoluble, and it is a member of the steroid hormone family. Production of 25(OH)D occurs mainly via the skin when exposed to ultraviolet-B. It also produced particularly as a result of food intake [4]. Although the role of 25(OH)D in the musculoskeletal system is well-known, recent studies have reported its effects on the endocrine, cardiovascular, immune and neurological systems. Additionally, it has various biological functions, such as cell differentiation, apoptosis and anti-inflammatory effects [5]. 25(OH)D regulates its role via the vitamin D receptor (VDR) located in the cell nucleus which controls both genomic and non-genomic pathways [6]. When the active metabolite 1, 25$(OH)_2D_3$ binds to VDR in the target tissue, physiological effects of 25(OH)D occur. Germ cells, spermatozoa, Leydig cells and epithelial cells lining the male reproductive tract express VDR and 25(OH)D metabolizing enzymes [7]. This expression has direct effects on spermatogenesis, sex hormone production and sperm maturation. Several animal studies reported that decreased serum 25(OH)D levels were associated with impaired sperm quality and fertility [7–9]. Another animal study reported that VDR regulates the synthesis of oestrogens in the reproductive system; this is an

essential factor in sperm quality [10]. Although there is currently no consensus with regard to the association between serum sex steroids and serum 25(OH)D levels, animal studies reported that serum 25(OH)D has a stimulatory effect on serum testosterone levels [10,11]. However, another study reported that there was no relationship between serum 25(OH)D and serum testosterone levels [12].

To date, several animal and human studies have investigated the relationship between serum 25(OH)D levels and male infertility. Despite this fact, the association between serum 25(OH)D and idiopathic male infertility is still controversial. In the study, we aimed to investigate the association between serum 25(OH)D, sex hormones and sperm quality in patients with idiopathic male infertility.

2. Material and methods

Between June 2018 and June 2020, 297 men were enrolled in this cross-sectional, observational, retrospective study in Izmir Bakırçay University and Recep Tayyip Erdogan University Urology Department. A total of 147 participants were male patients with idiopathic male infertility. The remaining 150 participants were fertile men. Detailed histories were recorded and physical examinations were performed. Blood samples were collected from the antecubital vein between 8:00–10:00 AM. These samples including serum sex hormones, serum 25(OH)D levels, glucose tests, lipid profiles, liver function tests and kidney function tests. At least two sperm analyses were performed on all participants in the study. Scrotal Doppler ultrasonography evaluation and karyotype analysis were performed on all of the patients. All patient demographic, laboratory and radiological features were recorded for statistical analysis.

Patients were divided into two groups. The first group (study group) included patients with idiopathic infertility. The second group (control group) included fertile patients who had a normal spermiogram examination and/or had a child. Demographic, laboratory and radiological features were compared statistically between groups.

The following exclusions were included in the study's exclusion criteria: the presence of uncontrolled diabetes mellitus, uncontrolled hypertension, uncontrolled lipid metabolism disorders, neurological diseases, haematological diseases, urinary tract infection, malignancies, chronic kidney failure, metabolic syndrome, psychiatric diseases and/or medical treatment, smoking, varicoceles, genetic disorders (karyotype disorders, AZFa/b/c gene mutations), history of varicocelectomy, hydrocelectomy, testicular sperm extraction (TESE) or vas deferens surgery, cardiac surgery, pelvic radiotherapy, medication which interfere with serum 25(OH)D level or spermatogenesis and patients with severe oligospermia (sperm concentration lower than 5 million/mL), azoospermia and teratozoospermia.

Sperm conduction was performed by masturbation in accordance with the procedure recommended by the World Health Organisation (WHO) 2010. In accordance with the WHO (2010) criteria, sperm samples with normal values for semen volume, sperm count, motility, vitality and morphology were included the study.

Serum 25(OH)D levels were analysed with a chemiluminescence assay (ADVIA Centaur XP®, Siemens, Tokyo, Japan). The intra-assay coefficients of variation were 11.9% at a 25(OH)D concentration of 13.6 ng/mL; 9.9% at a 25(OH)D concentration of 17.2 ng/mL; 7.2% at a 25(OH)D concentration of 28.2 ng/mL; 6.1% at a 25(OH)D concentration of 46.1 ng/mL; 6.0% at a 25(OH)D concentration of 73.2 ng/mL and 4.2% at a 25(OH)D concentration of 114.1 ng/mL. The test's reference range was 4.2 to 150 ng/mL (10.5–375 nmol/L). The test's accuracy was 0.99 (ID - LC/MS/MS) + 0.53 ng/mL (r = 0.96), and its detection limit was 3.20 ng/mL (8.0 nmol/L).

All statistical analyses were conducted by SPSS Statistics 26.0 (IBM Inc., Chicago, IL, USA) package program. Categorical variables were described by frequencies and percentages; continuous variables were described by means and standard deviations. The Kolmogorov-Smirnov test was used to evaluate the normality of the distributions. The Mann Whitney-U test was used to compare groups and quantitative independent data. The Chi-square test was used for qualitative independent data. Spearman's correlation analysis was applied for correlation. Significant results were analysed and investigated further using the logistic regression test. This was done after age, body mass index (BMI) and smoking were adjusted as confounders, ensuring that the findings were not biased by confounding factors. A p-value of less than 0.05 was chosen as the criterion for statistical significance.

3. Results

The mean age of the patients was 31.98 $\pm$ 6.97 years and mean Body Mass Index (BMI) was 24.84 $\pm$ 4.24 kg/m^2. A total of 95 patients (32%) were smokers and 4 patients (1%) suffered from alcohol addiction. The patient demographic and laboratory characteristics were summarized in Table 1. The mean serum 25(OH)D level of the patients was 23.16 $\pm$ 10.40 ng/dL. The mean infertility duration of idiopathic infertile men was 29.88 $\pm$ 28.86 months. Semen analyses of idiopathic infertile men and fertile men were summarized in Table 2. With regard to the Spearman's correlation analysis, we observed a positive correlation between serum 25(OH)D levels and infertility duration (r = 0.118; p = 0.048), total testosterone (r = 0.200; $p <$ 0.001), total sperm concentration (r = 0.163; p = 0.005), total sperm motility (r = 0.118; p = 0.042), progressive sperm motility (r = 0.119; p = 0.041) and negative correlation between Folicular Stimulating Hormone (FSH) (r = –0.150; p = 0.010), Luteinizing Hormone (LH) (r = –0.188; $p <$ 0.001), sperm morphology (r = –0.190; $p < 0.001$) in patients with idiopathic male infertility. Correlation coefficient ratio of Spearman's rank test was summarized in Table 3. According to the univariate analysis, statistical sig-

Table 1. Patient demographic and laboratory characteristics according to the groups.

	Infertile group	Fertile group	*p*-value
	n = 147	n = 150	
Age, mean, year	31.80 ± 7.00	32.15 ± 6.95	0.796
BMI, kg/m^2	26.44 ± 3.55	27.22 ± 4.22	<0.001
Glucose, mean, mg/dL	94.11 ± 12.41	99.31 ± 14.20	<0.001
Total cholesterole,mean, mg/dL	187.65 ± 38.39	207.44 ± 42.96	<0.001
Triglyseride, mean, mg/dL	159.07 ± 87.26	164.32 ± 84.19	0.486
HDL, mean, mg/dL	44.36 ± 10.07	47.57 ± 9.84	0.002
LDL, mean, mg/dL	117.60 ± 38.37	128.21 ± 37.33	0.006
AST, mean, IU/L	23.73 ± 14.54	21.77 ± 8.12	0.670
ALT, mean, IU/L	30.86 ± 28.67	24.57 ± 12.69	0.180
Calcium, mean, mg/dL	9.78 ± 0.37	9.52 ± 0.80	<0.001
Phosphore, mean, mg/dL	3.34 ± 1.72	3.32 ± 0.67	0.081
Vitamin D, mean, ng/mL	25.95 ± 10.97	21.37 ± 9.73	<0.001
CRP, mean, mg/L	0.52 ± 0.93	1.15 ± 2.67	0.651
Total protein, mean, g/dL	7.60 ± 0.39	7.47 ± 0.43	0.005
Albumin, mean, g/dL	4.61 ± 0.26	4.41 ± 0.29	<0.001
Total testosterone, mean, ng/mL	4.40 ± 2.00	4.84 ± 2.01	0.035
Oestradiol, mean, pg/mL	29.50 ± 9.88	33.01 ± 8.51	<0.001
FSH, mean, mIU/mL	5.01 ± 4.28	6.38 ± 8.01	<0.001
LH, mean, mIU/mL	4.29 ± 1.83	5.70 ± 6.30	<0.001
Prolactine, mean, pg/mL	8.89 ± 2.42	9.28 ± 9.82	0.002

Table 2. Semen analyse characteristics of patients according to the groups.

	Infertile group	Fertile group	*p*-value
	n = 147	n = 150	
Total sperm count, ×10^6	41.95 ± 46.26	57.83 ± 25.10	<0.001
Total sperm motility (a + b), %	37.65 ± 18.83	61.23 ± 9.77	<0.001
Progressive motile sperm (a), %	29.69 ± 17.64	45.37 ± 6.29	<0.001
Normal sperm morphology, %	12.92 ± 8.10	21.29 ± 5.37	<0.001

nificance existed between groups in terms of BMI, serum glucose, total cholesterol, HDL, LDL, 25(OH)D, total protein, albumin, oestrogen, FSH, LH, prolactin, total testosterone, total sperm concentration, total sperm motility, progressive sperm motility and normal sperm morphology. After age, body mass index (BMI) and smoking were adjusted as confounders on logistic regression analysis, we found that serum 25(OH)D was an independent risk factor for total sperm motility, progressive sperm motility and impaired sperm morphology. Table 4 summarized the logistic regression analysis results. Although statistical significance was observed between serum 25(OH)D levels and serum total testosterone, FSH levels and LH levels in multivariate analyses, we could not observe a significant association between serum 25(OH)D levels and serum testosterone, FSH levels and LH levels. On multivariate analyses, we observed that decreased 25(OH)D levels were associated with increased BMI (Odd ratio (OR) = 0.95), increased serum glucose levels (OR = 1.03), decreased serum albumin levels (OR = 0.01), decreased total sperm motility (OR = 1.21), decreased progressive sperm motility (OR = 0.89), and impaired sperm morphology (OR = 1.17).

4. Discussion

This cross-sectional, observational, retrospective study revealed that low serum 25(OH)D levels were correlated with impaired sperm quality. Although no association was found between serum 25(OH)D levels and total sperm concentration, there was a positive correlation between serum 25(OH)D levels, impaired sperm morphology, total sperm motility and progressive sperm motility. Additionally, decreased serum 25(OH)D levels were not a risk factor for hypogonadism. No association was found between serum 25(OH)D levels and serum total testosterone levels, FSH levels and LH levels. However, serum

Table 3. Spearman's Correlation Coefficient ratio of patients with idiopathic male infertility.

	Vitamin D	FSH	LH	Estradiol	Prolactine	Testosterone	Total sperm count	Total sperm motility	Progressive sperm motility	Sperm morphology
Vitamin D		–0.150**	–0.188**	0.014	–0.039	0.200**	0.163**	0.118*	0.119*	–0.190**
FSH			0.713**	0.134*	0.010	0.114	–0.053	0.178**	0.107	0.089
LH				0.180**	0.059	0.165**	0.057	0.189**	0.097	0.127*
Estradiol					0.024	0.168**	0.013	0.180**	0.122*	0.095
Prolactine						–0.069	–0.124*	–0.139*	–0.152**	–0.138*
Testosterone							0.053	0.176**	0.167**	0.037
Total spearman's count								0.544**	0.516**	0.419**
Total sperm Motility									0.915**	0.453**
Progresssive sperm motility										0.409**

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

Table 4. Univariate and Multivariate analyse results of the groups, after age, smoking and BMI were adjusted as confounding factors.

	Univariate analyses	Multivariate analyses	
	p-value	OR (95% CI)	*p*-value
BMI	<0.001	0.95 (0.90–1.00)	<0.001
Glucose	<0.001	1.03 (1.00–1.07)	0.036
Total cholesterole	<0.001	1.02 (0.99–1.06)	0.133
HDL	0.002	1.01 (0.95–1.07)	0.703
LDL	0.006	0.97 (0.94–1.00)	0.139
Vitamin D	<0.001	0.95 (0.90–1.00)	0.048
Total protein	0.005	1.65 (0.38–7.05)	0.496
Albumin	<0.001	0.01 (0.02–0.16)	<0.001
Oestradiol	<0.001	1.00 (0.96–1.05)	0.198
FSH	<0.001	1.05 (0.88–1.25)	0.530
LH	<0.001	1.20 (0.86–1.67)	0.264
Prolactine	0.002	1.10 (0.95–1.27)	0.198
Total testosterone	0.035	1.25 (0.93–1.68)	0.131
Total sperm concentration	<0.001	1.03 (0.99–1.17)	0.614
Total sperm motility	<0.001	1.21 (1.12–1.30)	<0.001
Progressive sperm motility	<0.001	0.89 (0.82–0.96)	<0.001
Sperm morphology	<0.001	1.17 (1.09–1.26)	<0.001

25(OH)D levels were associated with low serum albumin levels. We consider that these results may contribute to decreased male fertility rates by decreasing free testosterone levels, or the active form of testosterone *in vivo*.

The role of 25(OH)D in male fertility appears to be predominantly due to its effect on testicular function. The testicular function consists of two interconnected and complementary processes, hormone production and spermatogenesis; in coordination with the action of the accessory glands, these processes ensure the proper potential of male fertility [13]. Spermatogenesis encompasses a complex network of events that occur in the seminiferous tubules. This network includes: spermatocytogenesis, including the proliferation of spermatogonia and differentiation in spermatocytes; spermatogenesis, which represents the meiotic division of spermatocytes with the production of spermatids; spermiogenesis, which includes the stages of maturation and differentiation of spermatids in mature spermatozoa and spermiation, which consists of the release of mature spermatozoa into the lumen of the seminiferous tubules [14]. Testosterone is the primary male sex hormone and an essential anabolic hormone that plays a central role in the development and function of the male reproductive system and the regulation of sexual function; additionally, it contributes to the health of the musculoskeletal system and is involved in many important processes, including metabolism, cognition and mood [15]. Testosterone releases from the Leydig cell in the testis and is regulated by pulsatile LH release from the hypothalamus, autocrine and paracrine signals provided by growth factors and cytokines secreted in the testis [16]. In the Bellastella study, 122 men with type 2 diabetes were compared to 51 hypogonadotropic and 71 normo-gonadotropic men; it was reported that men with hypogonadism had lower serum testosterone levels than normo-gonadotropic men [17]. In Wehr's large population human study including 2299 men, it was reported that men with serum 25(OH)D levels above 30 ng/dL had higher serum testosterone levels than patients whose serum 25(OH)D levels were under 30 ng/dL [18].

On the other hand, several observational studies reported that serum 25(OH)D levels were not associated with circulating levels of total and free testosterone [19–21]. Similarly, Blomberg's recent study investigated young infertile men, and it was revealed that serum 25(OH)D levels were not correlated with serum total testosterone levels; a positive association, however, was found with sex hormone-binding globulin and a negative correlation was found with serum-free testosterone levels. In our study, it was revealed that decreased serum testosterone levels were not associated with lower serum 25(OH)D levels in men with idiopathic male infertility. Impaired semen quality may be related to sex hormone-binding globulin or free testosterone levels. Although we could not measure the sex hormone-binding globulin, we observed a positive correlation between serum 25(OH)D levels and albumin levels; these levels have similar roles to sex hormone-binding globulin. We observed low albumin levels in patients with low serum 25(OH)D levels, and it was found that these levels may be related to low sex hormone-binding globulin and low free-testosterone levels.

Recently, both animal and human studies have investigated the role of 25(OH)D on the reproductive system. Since 25(OH)D carries out its effects via VDR, several studies investigated VDR in the male reproductive system. These studies showed that, in the male gonads, both VDR and its metabolizing enzymes are expressed in the male reproductive tract, such as sertoli cells, germ cells, Leydig cells, spermatozoa, and the epithelial lining of the ducts [22,23]. The expression of VDR in sperm is predominantly localized in the head and nucleus. Due to this localization of VDR in sperm, some studies associate low 25(OH)D levels with impaired sperm quality. VDR mediates a non-genomic increase in intracellular calcium concentration and sperm motility, and it induces the acrosome reaction. In an animal study, Oury [24] reported that the loss of 25(OH)D regulated transcellular calcium transporter TRPV6 results in impaired sperm motility and infertility. CYP2R1, CYP27B1 and CYP24A1 are the 25(OH)D metabolising enzymes that are localized in the testis, epididymis, seminal vesicle, prostate and spermatozoa of humans [25]. The local expression of 25(OH)D metabolising enzymes is important for the regulation of intracellular 1,25-hydroxy vitamin D, which is active form of 25(OH)D and VDR activation [7]. In a cross-sectional study evaluating healthy men from the general population, 25(OH)D deficiency was associated with reduced sperm motility and the percentage of normal morphology [26]. Another study comparing 195 fertile men to 364 infertile men reported that infertile men had significantly lower serum testosterone levels and low serum 25(OH)D levels; these levels were associated with impaired sperm quality in terms of sperm motility and morphology [27]. A similar study by Tirabassi reported that low serum 25(OH)D levels were an independent risk factor for impaired total sperm motility and progressive sperm motility [28]. In our study, we revealed a positive correlation between total and progressive sperm motility, similar to previous studies. However, low serum 25(OH)D levels were not associated with sex hormone levels. Additionally, sperm morphology was correlated with 25(OH)D deficiency.

This cross-sectional, observational, retrospective study also has some limitations. First, serum sex hormone-binding globulin and free testosterone levels were absent. For this reason, we hypothesise that low serum 25(OH)D levels may impair semen quality via decreasing sex hormone-binding globulin and free testosterone levels, as these are more potent than the total testosterone in the male reproductive system and spermatogenesis. The second limitation is that we could not compare the seasonal difference of serum 25(OH)D levels, and this may be effective with regard to infertility. The last limitation of the study was that the paternity rates after 25(OH)D supplementation could not be observed. This treatment may provide the basis for supportive data with regard to our hypothesis.

In this study, we consider that 25(OH)D deficiency may cause deterioration of sperm quality in many genomic and non-genomic pathways. Low levels of 25(OH)D may reduce intracellular calcium concentration, which may decrease the effect of 25(OH)D through non-genomic pathways. As a result, 25(OH)D deficiency may cause the deterioration of sperm motility. This deterioration in sperm can occur in similar ways in the epididymis, which is vital for sperm maturation.

5. Conclusions

Recently, there are still controversial results regarding the aetiology of idiopathic male infertility, particularly those considering high and increasing rates. According to recent animal and human studies, serum 25(OH)D levels play a role in the male reproductive system, especially in spermatogenesis and sperm maturation. In this study, we observed a positive correlation between serum 25(OH)D levels, total sperm motility, progressive sperm motility and negative association with impaired sperm morphology. Serum testosterone levels were not correlated to serum 25(OH)D levels. 25(OH)D supplementation therapy may result in beneficial outcomes in patients with idiopathic male infertility.

Author contributions

Study design—MOH; Data collection—MOH, AC, HE; Statistical analysis—MOH; Manuscript writing—MOH, AC, YI, HE; Manuscript editing—MOH, AC, YI. All authors contributed to editorial changes, read the study and approved its content.

Ethics approval and consent to participate

This cross-sectional retrospective study was performed between June 2018 and June 2020 at the Bakircay University Cigli Training and Research Hospital and the Recep Tayyip Erdoğan University's Urology Department. The study protocol was reviewed and approved by the committee of Bakircay University (ethic committee number: 2017/56). This study enrolled patients with idiopathic male infertility as a study group and fertile patients as a control group. All participants gave their informed consent for inclusion prior to participating in the study.

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Conflict of interest

The authors declare no conflict of interest.

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