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ASSOCIATION OF 25-HYDROXY-VITAMIN D LEVELS WITH MALE REPRODUCTION PARAMETERS

Anfal BELKACEM[1,2], Soumia ZEGGAI[2-3], Siheme OUALI[1-2]✉, Noria HARIR[1,2], Khalida ZEMRI[1,2], Amira MESSAOUD[1,2], Feriel SELLAM[4], Chahnaz HADJSLIMANE MIRALI[5], Feryel SENOUSSAOUI[6]

[1] Biology Department, Faculty of Natural Sciences and Life, Djillali Liabés University of Sidi-Bel-Abbés, Algeria
[2] Laboratory of Molecular Microbiology, Health and Proteomics, Faculty of Natural Sciences and Life, Djillali Liabés University of Sidi-Bel-Abbés, Algeria
[3] Biology Department, Faculty of Sciences M'hamed Bougara, University of Boumerdes, Algeria
[4] National Research Center of Biotechnology CRBT, Constantine, Algeria
[5] Centre El assrar, Oran, Algeria
[6] Medically Assisted Procreation, AL ANIS Clinic, Oran, Algeria

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Abstract

Introduction. Recently, the question of whether vitamin D exerts an effect on the pathogenic process of infertility has become the focus of attention because the vitamin D receptor and the enzymes that metabolize vitamin D are expressed in both male and female reproductive cells.
The objective of the study was to investigate the impact of hypovitaminosis D on male fertility in the Tiaret region (western Algeria).
Material and methods. A prospective epidemiological study was conducted on 363 infertile men in the infertility clinic (Tiaret region) from May to September 2023. The participants were categorized into two groups according to 25-hydroxy vitamin D levels (deficiency<20 ng/mL and normal >30 ng/mL).

Résumé

Association des taux de 25-hydroxyvitamine D avec les paramètres de la reproduction masculine

Introduction. Récemment, la question si la vitamine D exerce un effet sur le processus pathogène de l'infertilité est devenue le centre d'attention, car le récepteur de la vitamine D et les enzymes qui métabolisent la vitamine D sont exprimés dans les cellules reproductrices masculines et féminines.
L'objectif de cette étude était d'étudier l'impact de l'hypovitaminose D sur la fertilité masculine dans la région de Tiaret (ouest de l'Algérie).
Matériel et méthodes. Une étude épidémiologique prospective a été menée auprès de 363 hommes infertiles dans une clinique spécialisée (région de Tiaret) entre mai et septembre 2023. Les participants ont été

✉ Address for correspondence: Siheme OUALI
Biology Department, Faculty of Natural Sciences and Life, Djillali Liabés University of Sidi-Bel-Abbés
Address: BP No. 89 Sidi-Bel-Abbés, 22000 Algeria
Email: oualisihem16@gmail.com

Results. 25-hydroxyvitamin D deficiency was noted in most of our patients (78.2%). Evidence of association between age, residence, body mass index, duration of infertility and vitamin D status was noticed (p<0.001). We found a negative correlation between vitamin D level and duration of infertility (p<0.0001). Luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone levels had a significant correlation with vitamin D deficiency (p<0.001). Moreover, sperm analysis results revealed a high frequency of pathological spermograms such as asthenospermia, azoospermia, oligospermia and teratospermia in patients with 25-hydroxy vitamin D deficiency.
Conclusions. The results of our study showed that 25-hydroxyvitamin D deficiency had an impact on male fertility as well as on sperm quality and testicular and pituitary hormones.

Keywords: 25-hydroxy vitamin D deficiency, male fertility, hormones, infertility duration.

List of abbreviations:
BMI = body mass index
FSH = follicle-stimulating hormone
IBM = International Business Machines Corporation
LH = luteinizing hormone
P = probability value
SD = standard deviation
SPSS = Stands for Statistical Package for the Social Sciences
VDR = vitamin D receptor
25(OH)D3 = 25-hydroxyvitamin D3
1,25(OH)2D3 =1,25-dihydroxyvitamin D3

répartis en deux groupes en fonction de leur taux de 25-hydroxyvitamine D (carence < 20 ng/ml et taux normal > 30 ng/ml).
Résultats. Une carence en 25-hydroxyvitamine D a été observée chez la majorité de nos patients (78,2%). Une association entre l'âge, le lieu de résidence, l'indice de masse corporelle, la durée de l'infertilité et le statut en vitamine D a été mise en évidence (p < 0,001). Nous avons constaté une corrélation négative entre le taux de vitamine D et la durée de l'infertilité (p < 0,0001). D'autre part, les taux sériques d'hormone lutéinisante (LH), d'hormone folliculo-stimulante (FSH) et de testostérone présentaient une corrélation significative avec les groupes de vitamine D (p < 0,001). De plus, les résultats de l'analyse du sperme ont révélé une fréquence élevée de spermogrammes pathologiques tels que l'asthénospermie, l'azoospermie, l'oligospermie et la tératospermie chez les patients présentant une carence en 25-hydroxyvitamine D.
Conclusions. Les résultats de notre étude montrent que la carence en 25-hydroxyvitamine D a eu un impact sur la fertilité masculine ainsi que sur la qualité du sperme et les hormones testiculaires et hypophysaires.

Mots-clés: carence en 25-hydroxyvitamine D, fertilité masculine, hormones, durée de l'infertilité

Introduction

Worldwide, more than 186 million people suffer from infertility, the majority of them residents in developing countries[1-2]. The etiology of infertility is attributed to several factors; in nearly 30% of couples, male reproductive abnormalities were the responsible factors[3-4].

Recently, the question of whether vitamin D exerts an effect on the pathogenic process of infertility has become the focus of attention, because vitamin D receptor (VDR) and the enzymes that metabolize vitamin D are expressed simultaneously in Sertoli cells, germ cells, Leydig cells, spermatozoa and in the epithelial cells lining the male reproductive system. The presence of vitamin D-metabolizing enzymes suggests that reproductive organs may modulate local vitamin D response in animals and humans. Testicular somatic or germ cells appear capable of synthesizing and locally degrading vitamin D, independent of systemic vitamin D metabolism. Moreover, the VDR expression in the testes suggests that vitamin D may exert both autocrine and paracrine actions and may play a role in regulating testicular function[5]. Some studies have shown that serum 25-hydroxyvitamin D3 [25 (OH)D3] is positively associated with sperm motility *in vitro*. In addition, 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] has been shown to increase intracellular calcium concentration in human spermatozoa, thereby improving sperm motility and acrosome reaction[6]. This suggests that hypovitaminosis D has a negative impact on sperm and hormone function, both in animals and humans[7]. Nevertheless, a consensus on the role of vitamin D in male fertility is still being debated.

The objective of the study was to explore the impact of hypovitaminosis D on male fertility, as well as on seminal parameters, testicular hormones, and pituitary hormones in the Tiaret region (western Algeria).

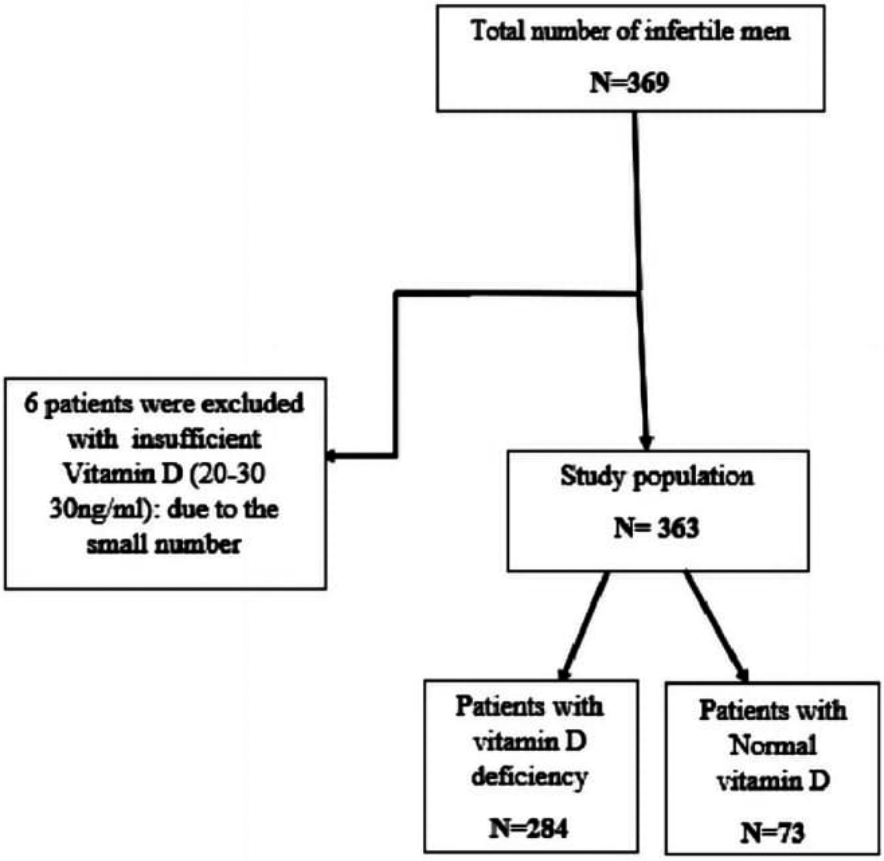

Figure 1. Flow chart of study population

Material and methods

The research was designed as a prospective study conducted from May to September 2023 on 369 infertile men admitted to the fertility clinics EL-ASSRAR Center and Al-ANIS Clinic, Oran (western Algeria). All the included patients were revised for the following criteria: hormonal profile, sperm status, anthropometric factors, age, residence and 25-hydroxyvitamin D levels.

The participants were categorized into 3 groups according to 25-hydroxy vitamin D levels: deficiency, insufficient and normal (<20 ng/mL, 20-30 ng/mL and >30 ng/mL, respectively). The patients of the insufficient group were excluded (six men) due to the small number of patients compared to the other 2 groups (deficiency and normal groups) (Fig. 1). Informed consent was obtained from all participants during the clinical consultation.

The men were included prospectively in the present study in accordance with the decree No. 387 (article 25) dated 31 July 2006 about ethical trials in Algeria and after approval from the Medical Committee of Biology department (Djillali Liabes University).

Statical analysis

The results were reported as frequencies (percentage) for qualitative data using Chi-square test and as mean ± standard deviation (SD) for continuous variables using Student t-test. Testosterone, FSH, LH ratios and infertility duration were compared according to serum 25-hydroxyvitamin level using Pearson correlation.

All the data were analyzed via Statistical Package for the Social Sciences (IBM Cop. Released 2017, IBM SPSS Statistics for windows, Version 25.0. Armonk, NY: IBM Corp). The criterion of statistical significance was p-value < 0.05.

Results

In our study, 363 infertile men were enrolled, with a mean age of 38.76±5.83 years. 25-hydroxyvitamin D deficiency (<20 ng/mL) was noted in 78.2% of our patients, while only 20.1% had normal vitamin D levels (≥30 ng/mL).

A significant relation was noted between age, residence, body mass index (BMI), duration of infertility and serum vitamin D level. The group most affected by vitamin D deficiency was aged between

Table 1. Relationship of vitamin D with age, BMI, area of residence, and infertility duration

		Vitamin D status		*p-value*
		Deficiency	*Normal*	
Age (years)	Mean±SD	38.72±6.12	39.73±4.39	0.187
	21-30 31-40 >40	27(9.5%) 139(48.9%) 118(41.5%)	12(16.4%) 0(0%) 61(83.6%)	<0.0001
BMI	Mean±SD	28.23±6.65	28.22±9.79	0.061
	18.5-24.9 25-29.9 >= 30	67(23.6%) 111(39.1%) 106(37.3%)	45(61.6%) 0(0.0%) 28(38.4%)	<0.0001
Area	Urbain Rural	164(57.7%) 120(42.3%)	55(75.3%) 18(24.7%)	0.006
Infertility duration (months)	Mean±SD	43.47±16.5	15.37±9.92	<0.0001
	<= 12 13-54 55-96	0(0%) 257(90.5%) 27(9.5%)	33(45.2%) 40(54.8%) 0(0%)	<0.0001

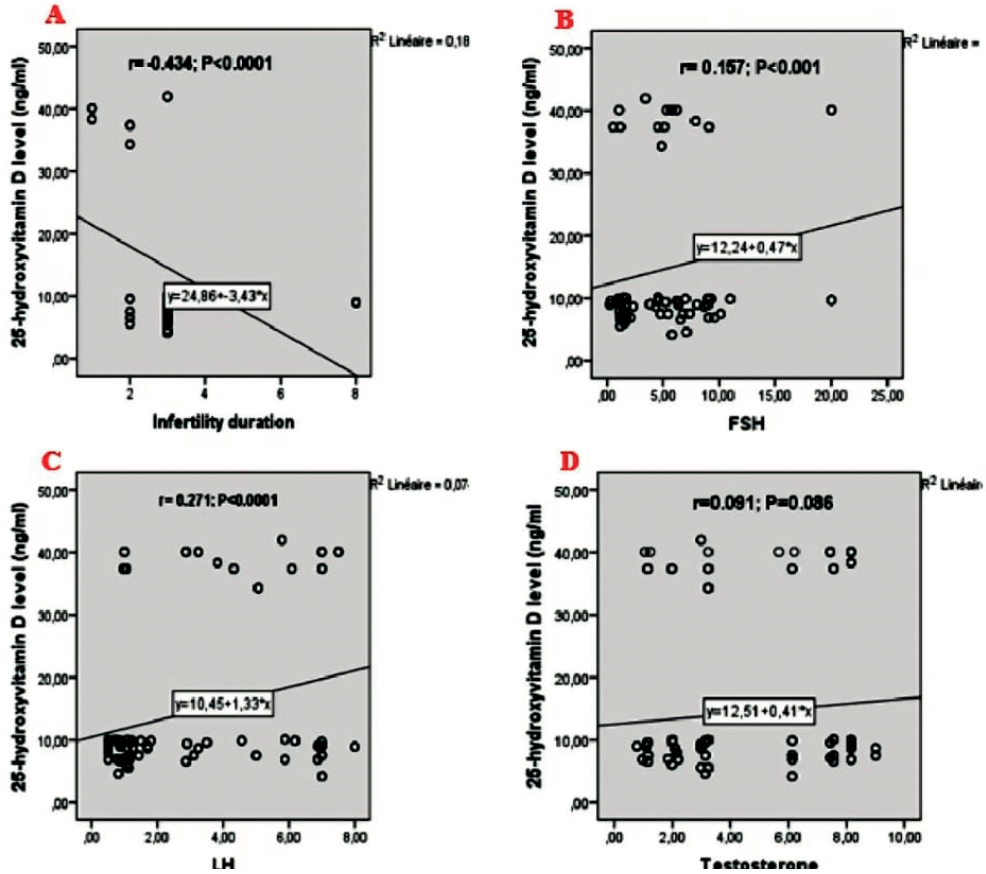

Figure 2. Correlation of 25-hydroxyvitamin D level with infertility duration, FSH, LH, and testosterone

31-40 years (48.9%), followed by men over 40 years (41.5%). Most obese and overweight men had hypovitaminosis D: 37.3% and 39.1%, respectively (p<0.0001). However, normal vitamin D levels were the highest in normal-weight men (61.6%).

More hypovitaminosis D cases were from urban area (57.7%) than rural area (42.3%) (Table 1). A negative correlation was noted between infertility duration and serum 25 OH-vitamin D level (Fig. 2). Moreover, men with vitamin D deficiency presented a long infertility duration (p <0.0001) (Table 1).

The hormonal profile analysis showed a significant relationship between FSH, LH, testosterone and serum 25-hydroxyvitamin D level. Furthermore, low FSH, LH and testosterone levels were more noticeable in the vitamin D-deficient group, in 46.5%, 44.7%

Table 2. Relationship of vitamin D with hormonal profile

		Vitamin D status		*p-value*
		Deficiency	*Normal*	
FSH	Mean±SD	4.5±3.85	5.98±4.87	0.006
	Low normal	132(46.5%) 152(53.5%)	15(20.5%) 58(79.5%)	<0.0001
LH	Mean±SD	2.68±2.46	4.4±2.23	<0.0001
	Low normal	127(44.7%) 157 (55.3%)	15(20.5%) 58(79.5%)	0.001
Testosterone	Mean±SD	4.77±2.77	5.29±2.66	0.148
	Low normal	188 (66.2%) 96(33.8%)	28 (38.4%) 45(61.6%)	<0.0001

Table 3. Relationship of vitamin D with spermogram

		Vitamin D status		*p-value*
		Deficiency	*Normal*	
Spermogram	Normal	27(9.5%)	41(56.2%)	<0.0001
	Asthenospermia	64(22.5%)	2 (2.7%)	
	Azoospermia	12 (4.2%)	1 (1.4%)	
	Oligospermia	94 (33.1%)	4 (5.5%)	
	Oligospermia+ Asthenospermia	33 (11.6%)	0(0%)	
	Teratospermia	54(19%)	25(34.2%)	

Table 4. Comparison of basic semen parameters in different classes of 25-hydroxivitamin D concentration

		Vitamin D status		*p-value*
		Deficiency	*Normal*	
Basic semen parameters	Volume mL	1.75±0.74	2.12±0.66	<0.0001
	Concentration (M/mL)	23.67±17.09	33.92±15.23	<0.0001
	Sperm total motility (%)	32.67±13.12	47.14±9.95	<0.0001
	Normal morphology (%)	3.58±1.18	4.54±1.05	<0.0001

and 66.2% of cases, respectively, while normal levels of these hormones were observed in most men with normal vitamin D status, at 79.5% for FSH and LH, and 61.6% for testosterone (Table 2).

As shown in Fig. 2, a positive correlation was observed between FSH, LH levels and serum 25-hydroxyvitamin D (p<0.0001).

Sperm analysis revealed a high frequency of pathological spermograms such as asthenospermia, azoospermia, oligospermia and teratospermia in patients with serum 25-hydroxyvitamin D < 20 ng/mL, respectively 22.5%, 4.2%, 33.1%, 19% (Table 3).

When subjects were classified according to serum 25-hydroxyvitamin D concentration, a significant difference was observed between the two groups in sperm concentration, sperm volume, total motility and normal morphology (Table 4).

Discussion

According to Muhittin et al.[8], the frequency of 25-hydroxyvitamin D < 25 nmol/L level was higher in patients aged 30-40 years, which is in line with our results. Therefore, daily vitamin D intake should be reassessed according to age[9], because body's need for vitamin D increases with age.

A significant relationship between hypovitaminosis D and higher BMI was noted in our research, which is similar to data from the literature[10-11].

Obesity is associated with an increased risk of low serum 25-hydroxyvitamin D concentrations[11]. Obesity epidemic is considered an important factor of hypovitaminosis D[12]. To explain this association between obesity and hypovitaminosis D, several mechanisms have been proposed, including limited sun exposure, poor diet or sequestration of vitamin D in subcutaneous fat deposits[13]. In addition, hypovitaminosis D has been implicated in several pathologies widespread in obese population, such as diabetes, metabolic syndrome, ischemic heart disease or stroke[14], highlighting the importance of optimizing vitamin D status, particularly in obese people[15].

Sun exposure induces the synthesis of vitamin D, which is mainly influenced by latitude and altitude of a region, season, time of exposure to the sun and ageing. During sun exposure, the 7-dehydrocholesterol present in skin absorbs ultraviolet B rays, then is converted into pre-vitamin

D3, producing photoproducts such as 25(OH)D3, which is metabolized in liver and kidneys, synthesizing vitamin D[16]. These data may explain our results concerning the impact of residence on serum vitamin D levels.

The significant relationship between 25-hydroxyvitamin D and the pituitary hormones FSH and LH can be explained by the presence of VDRs in the adenohypophysis and the fact that LH and FSH are produced by gonadotrophic cells of the pituitary gland[14]. Furthermore, it has been noted that C alleles of VDR can directly regulate the transcriptional activity of genes associated with LH synthesis[17]. It is therefore reasonable to state that VDRs may be involved in the production of these two pituitary hormones.

In our study, a positive association was observed between 25-hydroxy vitamin D and testosterone levels, which is similar with the results of other studies[18-19], that showed biological evidence of an association between vitamin D and male reproductive hormones. VDR and vitamin D-metabolizing enzymes have been shown to be expressed in human Leydig cells, where testosterone is synthesized in the male testicles[20], suggesting that vitamin D may have a direct effect on steroidogenesis.

Vitamin D may influence testosterone production via osteocalcin, produced by osteoblasts involved in bone metabolism. It has also been shown that vitamin D-induced osteocalcin expression may have a relevant indirect role in modulating testosterone production[7]. The presence of vitamin D3 receptors and vitamin D3-metabolizing enzymes in the testis, the ejaculatory system and mature spermatozoa could indicate the effect of vitamin D3 on spermatogenesis and maturation of human spermatozoa[21]. Evidence suggests that vitamin D deficiency may indirectly impair reproductive function via a calcium-dependent mechanism[20]. In addition, vitamin D has been shown to modulate cholesterol efflux, phosphorylation of tyrosine and threonine residues on specific proteins, and improve sperm survival and motility[22]. There is a growing body of evidence concerning the association of serum vitamin D levels with sperm quality and the functional significance of vitamin D[19, 23-24].

Conclusions

The results of our study show that vitamin D has a beneficial effect on male fertility and sperm quality. Testicular hormones may be affected by vitamin D levels. Because of the high incidence of vitamin D deficiency among Algerian men, the assessment of serum vitamin D concentration should be considered in the primary evaluation of male fertility. Vitamin D supplementation in men with low sperm quality should be considered and evaluated in the future.

Author Contributions:

Conceptualization, A.BEL., S. Z., S. O., and N.H. methodology, A.BEL., S. Z., S. O., and N.H., and A.B.; software, A.BEL., and S.O.; validation, A.BEL., S. Z., S. O., and N.H. ; formal analysis, A.BEL., S.O., and A.M; investigation, A.BEL., C.H., and F.S.; resources, A.BEL., C.H., and F.S.; data curation, A.BEL., S. O., S. Z., and N.H.; writing– original draft preparation, A.BEL., and S. O.,; writing–review and editing, A.BEL., S. Z., S. O., N.H., and F., Sel.; visualization, A.BEL., N.H., and K.Z.; supervision, N.H., K.Z.,; project administration, N.H., and K.Z. All the authors have read and agreed with the final version of the article.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article".

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2000(5), as well as the national law. Informed consent was obtained from all the patients included in the study".

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