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The association between serum vitamin D level and sperm parameters; A pilot study in a subset of Iranian infertile males

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Abstract

Introduction: Male-factor infertility affects about 7% of males in the general population. Vitamin D deficiency has been reported as an important public health issue all around the world and plays an important role in male fertility.
Objectives: The aim of the present study was to investigate the association between serum levels of vitamin D and sperm parameters.
Patient and Methods: We conducted this pilot cross-sectional study among infertile males who referred to Khanevadeh Specialty Hospital, Isfahan, Iran. A researcher-made checklist was used to collect data on basic characteristics of patients. Serum vitamin D level as well as sperm parameters (comprising sperm concentration, volume, vitality, total motility, progressive motility and abnormal morphology), DNA fragmentation and chromatin maturity were assessed according to standard protocols. Subjects with a serum vitamin D level of ≥30 ng/mL and 20-29 ng/mL were categorized as vitamin D sufficient and insufficient groups respectively.
Results: According to our results, sperm parameters except for sperm volume were significantly different between two groups ($P<0.05$). There was a positive significant correlation between sperm concentration, vitality, progressive motility and total motility with serum levels of vitamin D ($P<0.05$). A negative significant correlation was observed between sperm abnormal morphology, DNA fragmentation and chromatin immaturity with serum levels of vitamin D ($P<0.05$). We did not find any significant correlation between sperm volume and serum levels of vitamin D among infertile males ($P>0.05$).
Conclusion: The results of the current study showed that low vitamin D levels can be considered as a potential risk factor for male infertility among the Iranian population. Further large-scale studies are warranted to determine the association between male infertility with serum vitamin D level and underlying mechanisms.

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Introduction

Infertility is defined as the inability to conceive after one year of unprotected intercourse and has been considered as a major public health issue by world health organization (1). Male-factor infertility which is known as abnormal sperm parameters affects about 7% of males in the general population (2). It has also been reported that 20%-30% of infertility cases are the result of male factor alone (3). Various factors are known as predictors of male infertility comprising varicocele, systemic diseases, infections, genetic and lifestyle factors (4,5).

Increasing evidence from animal and human studies showed that nutrition is one of the most important lifestyle factors contributing to male infertility. In an animal study, Morgan et al found that consuming an enriched-cholesterol diet disrupts blood-testis barrier which is possibly linked to male infertility (6). The modification of testicular metabolism through consuming high energy diets has been known to decrease sperm quality. It has been assumed that the induction of mitochondrial dysfunction by consuming high energy diets is associated with oxidative stress and sperm defects (7). Additionally, accumulating evidence

Key point

This study is about the association of serum vitamin D level and sperm parameters including sperm concentration, volume, vitality, total motility, progressive motility, abnormal morphology, DNA fragmentation and chromatin maturity. In the current study we evaluated the association between serum vitamin D levels with sperm parameters, among a number of Iranian infertile males. Our results showed that all sperm parameters, except for sperm volume, were significantly correlated with vitamin D level.

indicated that supplementation with specific vitamins and micronutrients improves sperm quality and can be considered as a treatment option for male infertility (8).

Previous studies demonstrated that vitamin D plays an important role in male fertility according to the high expression of its receptors and metabolizing enzymes such as vitamin D receptor, cytochrome P450 2R1 (CYP2R1) and cytochrome p450 27B1 (*CYP27B1*) in male reproduction system (9). Some cross-sectional studies have found an association between the level of vitamin D and sperm parameters such as sperm morphology and motility (10-12). The results of a study by Yang et al on more than 500 males showed that serum vitamin D level is independently associated with sperm motility and morphology (10). The result was confirmed by another study which evaluated the association between vitamin D deficiency and sperm quality in 300 males from the general population (11). A negative association between both low and high vitamin D levels and sperm parameters has been reported by Hammoud et al (13). However, a number of studies have found no significant association between serum levels of vitamin D and sperm parameters (14). As a result, further large-scale studies are required to assess the association between vitamin D level with male infertility and underlying mechanisms.

Objectives

The objective of the present study was to investigate the association between serum levels of vitamin D and sperm parameters comprising traditional sperm parameters, sperm DNA fragmentation and chromatin maturity in a group of Iranian infertile males.

Patients and Methods

Study design and participants

This cross-sectional study was performed on infertile males aged 18 to 60 years who referred to Khanevadeh Specialty Hospital, Isfahan, Iran. The exclusion criteria were the presence of varicocele, systemic diseases, malabsorption, and malignancy and also vitamin supplements' intake. A researcher-made checklist was used to collect basic characteristics of participants comprising socio-demographic data, smoking status, opium intake, medical and infertility history.

Semen collection and analysis

The semen samples were collected after a 2 to 4- day period of masturbation abstinence. After complete liquefaction, standard semen parameters (volume, concentration, motility, vitality and abnormal morphology) were examined according to the World Health Organization (WHO) 2010 guidelines (15). Thereafter, a combination of two complementary assays, to assess sperm chromatin maturity (acidic aniline blue staining) and DNA fragmentation rate (sperm chromatin dispersion) were applied.

Sperm chromatin dispersion test

Sperm chromatin dispersion test was done according to a previously described protocol (16). A minimum of 200 sperm per sample were scored at ×1000 magnification of the light microscope (model, country). The percentage of sperms bearing DNA fragmentation was expressed as DNA fragmentation index (DFI) and was calculated as the percentage of sperm with small and no halos, over the total sperm count per slide.

Acidic aniline blue staining

Sperm chromatin maturity was tested using the SCMA kit (17). In the first step, 1×10^6 sperm/mL of each sample was centrifuged (300 g, 5 minutes). Then slides were prepared by smearing 10 µL of sperm suspension and the slides were allowed to dry in air and fix for 30 minutes at room temperature with a solution of 3% buffered glutaraldehyde. Slides were then stained through several steps of staining with aniline blue/eosin. For each stained smear, at least 200 spermatozoa were evaluated with a ×1000 magnification of a bright light microscope. Sperm heads with mature chromatin were stained pink while sperm heads with immature chromatin were stained blue. The results were presented as percentages of total chromatin immature sperm. An ejaculate having blue staining in <30% of sperm was considered normal.

Serum vitamin D levels

Fasting blood samples were collected from all patients and total concentration of vitamin D in serum was measured by HPLC (high performance liquid chromatography) method and classified as sufficient (≥30 ng/mL) and insufficient (20-30 ng/mL) according to the study by Holick (18).

Ethical issues

The research followed the tenets of the Declaration of Helsinki and its later amendments. Ethical considerations in this study included explaining the subject of research to individuals and obtaining written consent from them for the preparation of serum and sperm samples. Meanwhile, the subjects were assured that their information would be stored confidentially in information gathering forms and the results would be presented as a whole sample population. The present study was approved in the ethics committee of Esfahan University of Medical Sciences with registration number IR.MUI.MED.REC.1398.077. This study was a dissertation pathology residential thesis by Elham Omidi at this university (research project # 398048).

Statistical analysis

Quantitative and categorical variables were presented as mean±SD and frequency (percentage). Continuous normal variables were compared between two groups by using independent samples *t* test, while chi-square or Fisher's exact tests were used for categorical data. All statistical analyses were conducted using statistical package for the

social sciences (SPSS, version 21; SPSS Inc., Chicago IL). The correlation between serum levels of vitamin D with sperm parameters was assessed using Pearson's correlation analysis. $P<0.05$ was considered as statistically significant level.

Results

Baseline characteristics of subjects are summarized in Table 1. Totally, 70 participants comprising 49 (70%) vitamin D sufficient and 21 (30%) vitamin D insufficient infertile males included in the current cross-sectional study. Our results showed no significant difference between two groups in terms of basic characteristics ($P>0.05$). As shown in Table 2, two groups were significantly different regarding all sperm parameters except for sperm volume ($P>0.05$).

Results of the Pearson's correlation between serum vitamin D level with sperm parameters, chromatin immaturity and DNA fragmentation are presented in Table 3. We found a significant positive association between sperm concentration, progressive motility and total motility with serum vitamin D level ($r=0.05$, $P<0.001$; $r=0.49$, $P<0.001$; and $r=0.32$, $P<0.001$ respectively). However, there was a significant negative association between abnormal sperm morphology ($r=-0.57$, $P<0.001$), sperm DNA fragmentation ($r=-0.68$, $P<0.001$) and chromatin maturity ($r=-0.53$, $P<0.001$) with serum vitamin D level. Our results failed to show any correlation between sperm volume and the level of vitamin D.

Table 1. The comparison of basic characteristics between vitamin D deficient group with vitamin D sufficient group

	Sufficient vitamin D group (n=49)	Insufficient vitamin D group (n=21)	*P* value*
Age (years)	33.69 ± 5.66	36.33 ± 6.51	0.09
Employed (yes)	41.7	42.9	0.56
Abdominal exploration (yes)	4.1	14.3	0.15
Opium intake (yes)	12.2	9.5	0.5
Smoking (yes)	14.3	4.8	0.24
Duration of infertility (years)			
<3	87.2	70	0.08
>3	12.5	30	
Number of previous abortions			
<2	75	60	0.06
>2	25	40	
Number of children ever born			
<3	95	100	0.26
>3	5	0	
Sperm agglutination			
Normal	72.3	95.5	0.08
Abnormal	27.7	9.5	

*Resulted from chi-square test for categorical and independent samples *t* test or for continuous normal distributed variables.

Discussion

Vitamin D deficiency has been reported as an important public health issue all around the world. Vitamin D is best known for its role in bone health. However, recent studies have shown that inadequate vitamin D level is associated with the risk of various chronic diseases such as cardiovascular diseases, diabetes mellitus, respiratory disorders and malignancies (19,20). Moreover, a number of previous animal and human studies have shown that vitamin D is necessary for optimal function of male reproduction system (9,21). It seems that the effect of vitamin D on male fertility mediated through its role in spermatogenesis and the production of male reproductive hormones; however, further studies are warranted to detect underlying mechanisms precisely (22,23).

In the current study we evaluated the association between serum vitamin D level with sperm parameters, among a number of Iranian infertile males. Our results showed that all sperm parameters, except for sperm volume, were significantly correlated with vitamin D level. The finding is in accordance with previous studies. Tirabassi et al in a retrospective study on 104 subjects found that vitamin D levels were positively associated with both progressive and total sperm motility (24). The association between sperm parameters like sperm motility and morphology has also been reported by others (10,11). In contrast to our findings, several studies could not find any significant association between serum vitamin D levels and sperm parameters in males (13,14). The controversial results possibly are related to differences in study design and studied population. For example, the studied population in the present study was infertile males which their lifestyle, psychological and medical features are different from healthy subjects in

Table 2. The comparison of sperm parameters between vitamin D deficient group with vitamin D sufficient group

	Sufficient vitamin D group	Insufficient vitamin D group	*P* value*
Sperm volume (mL)	3.28 ± 1.43	3.13 ± 1.42	0.69
Sperm concentration (10^6/mL)	59.21 ± 31.63	28.23 ± 17.12	<0.001
Sperm progressive motility (% motile)	31.16 ± 5.16	18.52 ± 10.41	<0.001
Total sperm motility (% motile)	51.87 ± 8.45	41.19 ± 15.15	<0.001
Abnormal morphology (%)	95.76 ± 2.59	97.78 ± 0.73	0.001
Vitality (%)	86.28 ± 11.11	74 ± 21.57	0.002
DNA fragmentation score (%)	15.24 ± 4.69	23.76 ± 4.01	<0.001
Aniline blue staining (%)	23.55 ± 6.75	29.71 ± 5.29	<0.001

**P* values resulted from repeated measures ANOVA.
Note. Data are presented as mean ± standard deviation.

Table 3. Pearson's correlation coefficients between serum level of vitamin D and sperm parameters

	Sperm volume	Sperm concentration	Sperm progressive motility	Total sperm motility	Abnormal morphology	Vitality	DNA fragmentation score	Aniline blue-staining
Vitamin D level	0.06	0.5**	0.49**	0.32**	- 0.57**	0.26*	- 0.68**	- 0.53**

**$P<0.001$, *$P<0.05$.

previous studies. Thus, future case-control studies should compare the association between serum vitamin D levels with sperm parameters between healthy and infertile males considering confounding variables.

Sperm DNA fragmentation is known as one of the most important factors in the etiology of male infertility. According to previous research, sperm DNA fragmentation contributes to abnormal chromatin remodeling, apoptosis during sperm maturation and oxidative stress (25-27). Furthermore, some evidence has indicated that sperm chromatin maturity is associated with fertilization rate; as a result, its evaluation is of great importance in males' fertility assessments (28). The results of the current study showed a negative significant association between vitamin D level in serum with sperm DNA fragmentation and chromatin immaturity. It seems that vitamin D signaling affects gene expression in spermatozoa which consequently affects cellular and molecular events such as DNA fragmentation or chromatin maturity.

Conclusion

The results of the current study showed that serum vitamin D levels associated with various sperm parameters. Future large-scale interventional studies are required to investigate the impact of vitamin D supplementation on semen and hormonal parameters in infertile males.

Limitations of the study

This is a single center study. A multi-centric study with larger number of infertile patients is recommended in future studies.

Authors' contribution

MaryD and EO helped in the design of study and preparation of final draft. EO and MarzD contributed to data analysis. EO helped in conducting the study, data collection and doing interview and data analysis. MH helped in the design of study and preparation of final draft. All authors read and approved the final manuscript.

Conflicts of interest

The authors report no conflicts of interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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