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Assessment of Vitamin-D Levels Among Infertile Men in Iraq, A Comparative Study

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ABSTRACT

Background: Vitamin-D is one important steroid hormone in the body, which is obtained from direct sunlight and food or supplements.

Objective: The current study aimed to evaluate the relationship between Vitamin-D levels and semen parameters among infertile men in Najaf Governorate.

Patients and method: The study included sixty infertile men who presented to the Infertility Unit, Saad Medical City during the period from January 2020 to September 2020. The results were compared with the results of thirty healthy men as a control group. The level of Vitamin-D in the blood was detected using a French-origin Fujifilm machine, semen analysis, and sperm counting by using a Microcell counting chamber. Sperm morphology was evaluated by the Diff-Quick kit.

Results: The results demonstrated that infertile men had average age of 34 ± 0.50 years and their mean body mass index (BMI) was 60.60 ± 5.06 kg/m^2. The results showed that the level of vitamin-D in the body recorded a significant decrease (P-value < 0.0001) in infertile men (12.55 ± 0.02) as compared to fertile men (43.16 ± 0.005).

Conclusion: This study proved the positive relationship between the level of Vitamin-D and the levels of semen parameters, which can insure for the possibility of relying on the level of Vitamin-D in determining the cause of male infertility.

Keywords: Vitamin-D, Semen, Infertile, Men, Iraq.

INTRODUCTION

Infertility affects more than 186 million people in various countries of the world, especially developing countries [(1)]. The World Health Organization has identified infertility as a public health problem and considered it a disease that affects both sexes and leaves physical and psychological effects. Semen analysis is important in assessing the possibility of infertility [(2)]. Studies interested on this subject indicated a decrease in the percentages and concentrations of normal sperm in men of China, Brazil, North America, Europe, Australia, and New Zealand that maybe due to changes in lifestyle such as alcohol and tobacco use, diet, obesity, and other causes [(3).]

The major causes of infertility of males may be due to changes of semen parameters. Oligospermia is a lack in the sperm number in the semen of less than 15 million/milliliter, which is subdivided into light (10–15 million sperm/mL) severe (< 5 million sperm/mL) and moderate (5–10 million sperm/mL). Oligospermia is a conceder defect in sperm form or other causes like exposure to chemical or thermal factors that inactivated spermatogenesis, varicocele, hormonal factors, or idiopathic [(4)]. So, patients must be given guidelines to correct their lifestyle and prevent factors that affect spermatogenesis [(5)].

Approximately 10% of infertile men with oligospermia have non-normal karyotypes, suggesting that mutation of stem cells is responsible for sperm production disorder. So, men with severe oligospermia were treated using intracytoplasmic sperm injection (ICSI), which includes the direct injection of oocyte cytoplasm by single sperm [(6)]. Other causes may be due to Asthenozoospermia (AZS), diagnosed by lack of sperm motility where < 40% sperm motility or less than 32% with progressive motility. Low motility of sperm is a frequent escort of oligospermia and is often linked with a mixed image of appearance flaws that indicate faulty spermatogenesis [(7)].

The study of semen quality, the count and motility of sperm are the first diagnostic markers to be estimated. Sperm cell morphology (Teratospermia) is an undervalued men parameter, because of the loss of a general accepted estimated method though, the morphologic features of the sperm cell are the result of highly cellular modifications occurring through spermatogenesis [(8)]. The percentage of abnormal spermatozoa and specific structural abnormalities suggest a flaw related to spermatozoa formation and maturation of abnormal spermatozoa morphology that is associated with increased contemporary signs of sperm damage and decrease of semen quality parameters [(9)]. The current study aimed to evaluate the relationship between vitamin-D levels and semen parameters among infertile men in Najaf Governorate.

PATIENTS AND METHODS

The place and period of study: Al–Saader Medical city/Infertility Unite in Najaf Governorate, from January 2020 to September 2020.

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Healthy individuals and patients: Infertile males attending to Al–Saader Medical city /infertility Unite in Najaf Governorate, in addition to 30 healthy men as controls.

Inclusion criteria: Infertile Men.
Exclusion criteria: Fertile Men.
Study steps: Clinical examination, after that, take seminal fluid sample for diagnosis of semen parameters then draw a blood sample and isolate serum to measure the concentration of vitamin D.

Blood collection: The current study involved 60 infertile men their ages ranged from 25 to 48 years. The patients came to the Al Saadar Medical city Infertility Unit during the period from January 2020 to September 2020 in Al-Najaf Governorate. Five ml blood samples from each patient were withdrawn and put in dry and clean test tube. The samples were centrifuged at 3000 rpm for 5 minutes to get the serum, which is collected in another sterile tube. The samples were stored tightly closed at -20 ºC until measurement of vitamin D level [10, 11].

Semen analysis: After 2–3 days of abstinence from sex, samples of semen were collected by masturbation. After liquefaction, semen was thoroughly analyzed [12]. The number and motility percentages of sperm were determined using a MicroCell counting chamber (Vitrolife, San Diego, California), and all samples were examined by the same analyst.

Sperm morphology: After preparing thin smears from seminal fluid, stain the slides using a Dif-Quik kit (Baxter Healthcare Corporation, Inc., McGaw Park, IL, Company, China), classify the slides based on Kruger's strict criteria and determine a cutoff value according to the WHO 2010 guidelines. Using bright field illumination and an oil immersion objective with a magnification force of 2000, a specialized examiner examined all slides [13].

Measurement of vitamin D:

Vitamin-D concentration was measured by using the VIDAS technology and materials manufactured by company-r Eloile, France. Directly semen fluid was examined to determine semen fluid parameters oligospermia (number of sperm), asthenospermia (shape of sperm), and teratozoospermia (morphology of sperm) [14].

Ethical considerations:

The study concept for human studies was approved from Kufa University's College of Science and AL-Saddar Hospital by The Institutional Ethics Committee. Additionally, before taking part in the study, each individual gave written, informed consent.

Statistical Analysis

Windows 2010, SPSS version 23 was utilized to evaluate the study's data [15]. Unpaired sample t-tests was used to compare two groups, and one-way ANOVA test was performed to compare groups that had been separated based on the parameters that were assessed [16]. SPSS version 23 have been used to create each and every figure. Threshold of significance was set at $P \leq 0.05$ [17, 18].

RESULT

Age groups, Body Mass Index and smokers

The results demonstrated that infertile men had mean age of 34 ± 0.50 years compared to the control group (32 ± 0.57 years) **(Figure 1)**.

BMI was 57.40 ± 1.36 kg/m^2 in control and 60.60 ± 5.06 kg/m^2 in patients **(Figure 2)**. Also, the results of the current study indicated that there were 48.33% smokers and 51.67% were non-smokers compared to the control group (43.33% and 56.67% respectively) **(Figures 3)**.

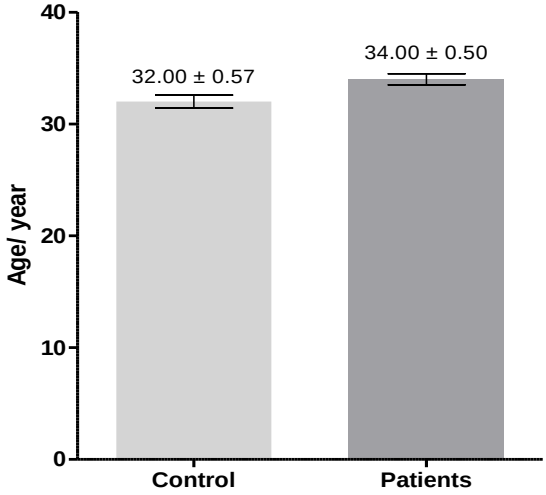

Figure (1): Mean ± standard error of age groups in healthy group and patients

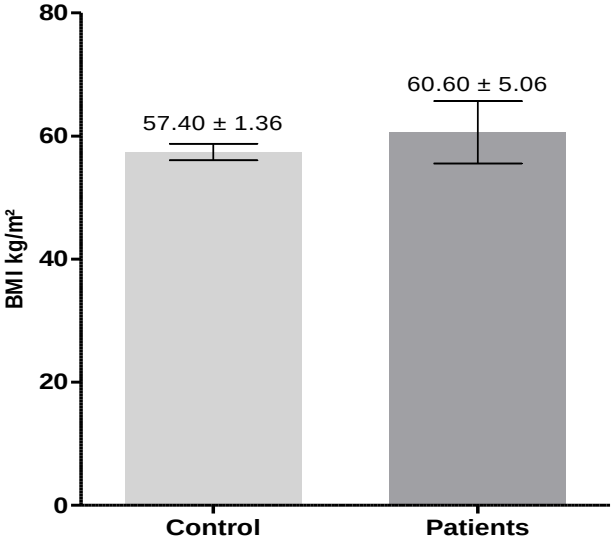

Figure (2): Body Mass Index (kg/m^2) of healthy group and patients

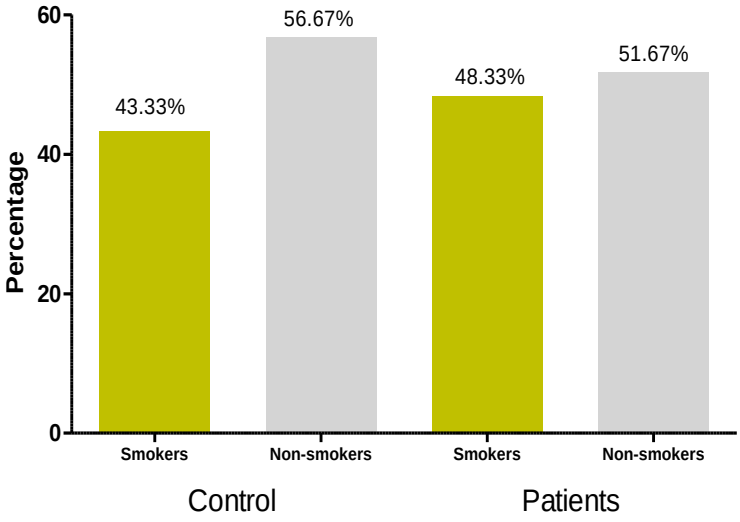

Figure (3): Compared between smokers and non-smokers of healthy group and patients.

Infertility and the level of Vitamin-D

The results of the current study showed the close relationship between male infertility and the level of vitamin-D in the body, as the study recorded a significant decrease (P-value < 0.0001) of vitamin-D in infertile (12.55 ± 0.02) as compared to fertile men (43.16 ± 0.005 pg/ml) **(Figure 4)**.

Also, the results showed that this deficiency affected semen parameters in varying proportions, some of which are related to the low number of sperm, which is known as oligospermia (23.38%), affection of the movement of sperm (asthenospermia, 21.35%), and what is related to the morphology of the sperm (Teratospermia, 16.26%) **(Figure 5)**.

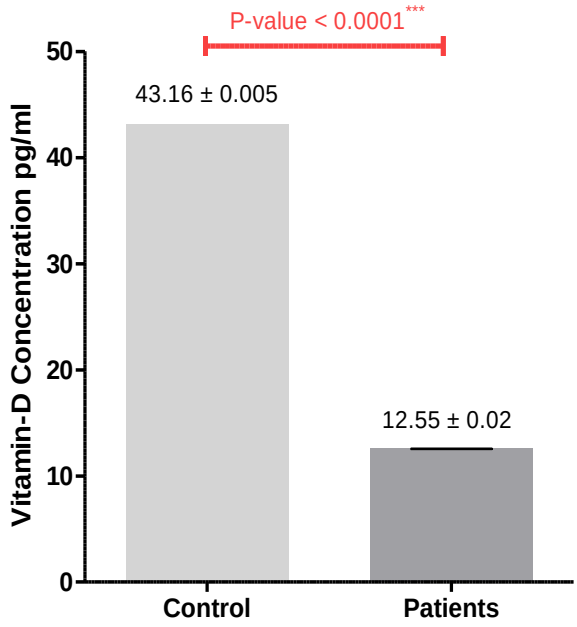

Figure (4): Vitamin-D concentration in healthy individuals and patients

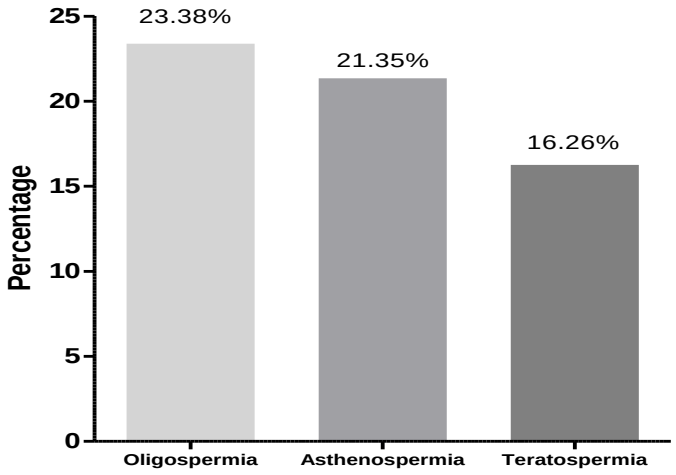

Figure (5): Percentages of Oligospermia, asthenospermia and teratospermia in patients

DISCUSSION

Vitamin-D levels and the quality of semen have been studied in humans, but the results were conflicting. Furthermore, there are few studies examining the relationship between vitamin-D and semen quality. This has prevented a decision regarding how much vitamin-D is optimally concentrated in a fertile reproductive system [19, 20]. Sperm kinetic parameters were positively correlated with Vitamin-D deficiency [7]. Vitamin-D was shown to positively affect sperm motion in men with idiopathic oligoasthenozoospermia and prostatitis [21]. It is believed that Vitamin-D plays a role in various reproductive organs and tissues, particularly those related to spermatogenesis and reproduction in men is not known. However, the specific mechanism by which vitamin-D affects male reproduction is not known [22]. The optimal function of sperm may rely on a direct effect of vitamin-D and may be affected indirectly via calcium homeostasis [23].

Laboratory studies showed the affirmative effect of Vitamin-D on spermatozoa [24]. According to one study, vitamin-D levels did not significantly affect sperm motility, but other studies showed that vitamin-D levels did positively influence sperm motility [25]. Fertilization is primarily a result of sperm motility, and improving sperm motility leads to better sperm reaching eggs to be fertilized and thus increasing childbearing, which in turn increases the chance of conception [26]. The seminal vesicles contribute to the production of 65-75% of semen [27]. Evidence has revealed that vitamin-D receptors and metabolic enzymes in the epithelial layer of human seminal vesicles contribute to this [28]. This indicates that vitamin-D plays an active role in the function of seminal vesicles and vitamin-D may enhance sperm motility by promoting activation of the mitochondrial respiratory chain in sperm [29]. For fertilization effectiveness to be effectively assessed, the three classical signals of semen quality (sperm motility, count, and morphology) must be strongly intertwined. It may be that a morphological flaw is more important in determining whether sperms are capable of fertilizing

effectively, if the concentration of motility is good [30] so, it is important to point out both the situation of morphology within the traditional parameters of semen in addition its situation as specific marker.

CONCLUSION

The current study reflected the positive relationship between the level of vitamin-D and the levels and quality of semen parameters, which assure the possibility of relying on the level of vitamin-D in determining the cause of male infertility.

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