Are serum vitamin D and ionized calcium linked with semen quality and sex steroid hormones in infertile men?

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Abstract

Malefactors contribute to nearly fifty percent of infertility cases. It is declared that serum vitamin D and serum ionized calcium (Ca+2) have an impact on male fertility by assisting in sperms maturation, motility, modulation of sperm survival, sex hormones production, and initiation of the acrosome reaction. The aim of the study was to investigate the association between vitamin D concentration, serum Ca+2, semen quality and levels of reproductive hormones in infertile men. A prospective study was undertaken involving 102 men. 52 men were assigned to the patient’s group included men with infertility. 50 men assigned as the control group included normal fertile men. Each participant in both groups provided double semen specimens for seminal fluid analysis with one fasting blood sample to measure serum vitamin D, serum Ca+2 and reproductive hormones. Data were collected and their relationships with each other were evaluated. Infertile men group had significantly lower vitamin D levels in comparison with the control group, but no difference in serum Ca+2 between the two groups was found. Infertile men with low serum vitamin D level had low total motile sperms, low progressive motile sperms and low sex hormone-binding globulin (SHBG), whereas no significant correlation with luteinizing hormone (LH), follicular stimulation hormone (FSH), estradiol (E2) and total testosterone (TT) was identified. Low level of serum Ca+2 was associated with low total motility, low progressive motility and exhibited a significant positive correlation with LH and FSH, but no correlation between TT, E2, SHBG and serum Ca+2 was recognized. In conclusion, serum vitamin D and Ca+2 were significantly linked to seminal fluid quality and some reproductive hormones in infertile men.

Key words: infertility, vitamin D, ionized calcium, reproductive hormones.

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Introduction

Infertility is identified when a sexually active, noncontracepting partners cannot conceive within one year in accord with the World Health Organization (WHO) 2000(1). A male contribution to infertility is found in 45–50% of the cases (2). The causes of diminished sperm production and activity may be linked with components working on pre-testicular, testicular or post-testicular levels. The cause is yet obscure in around 50% of patients’ so-called idiopathic infertility (3). Diagnosis includes a full history and physical examination, two to three analyses of semen and an endocrine profile. Genetic, another endocrine, and radiologic tests should be obtained when indicated (4). Vitamin D is a steroid hormone, its biological functions are attained through uniting 1, 25(OH)2D3 (active metabolite) to vitamin D receptor (VDR) which is dispensed through numerous organs (5).

In the testes VDR is found, enabling vitamin D to be an adjustable organizer of fertility manifesting a direct influence on the production of sex hormones and mature spermatozoa (6). More recently, it demonstrated an influence on gaining the sperms of its fertilization capability (7). Furthermore, insufficient vitamin D has been accompanied by deteriorated human semen quality. Subfertile patients with deficient vitamin D possessed lower sperm concentration and motility in comparison to men with normal vitamin D (8).

The calcium ion is essential in many biologic processes. In testis, Ca+2 is involved in genes reproduction specified for germ cells in males, meiosis as well as post-meiotic differentiation in addition to sperm action in fertilization in reaction to hormones along with local regulators. It has been demonstrated that the prostate, seminal vesicles, and epididymis are also very rich in calcium therefore several articles have investigated the relationship between calcium and infertility in men (9). Aim of the study: To investigate the association between vitamin D concentrations, serum ionized calcium, semen quality and levels of reproductive hormones in infertile men.

Patients and methods

A prospective study was conducted in Al- Yarmouk Teaching Hospital/department of urology from 1st January 2018 to 1st October 2019. Patients with a history of infertility (primary and secondary) referred to our consultation clinic were included in this study. At the clinical visit, detailed information was obtained concerning age, past illnesses, maturity, and fertility, the onset of seeking conception, co-morbidities, smoking, and history of mumps orchitis.

Physical examination was performed with scrotal ultrasound (U/S) to exclude varicocele and testicular atrophy. Weight, height, and body mass index (BMI) were measured. Informed consent was obtained from all patients then they were scheduled to deliver two semen samples with 14 days interval and one fasting blood sample for hormonal assay, vitamin D and ionized calcium measurements. Infertile males with varicocele, cryptorchidism, azoospermia, use of anabolic steroids or any medications that affect fertility, smoking, severe medical illness and
co-morbidities, urogenital infection, elevated serum prolactin, testicular atrophy, and trauma were excluded from the study.

The study comprised 52 patients with idiopathic infertility (group A) and 50 fertile men as a control group (group B). By masturbation, two semen specimens (with a 14-days interval) were obtained into a plastic can and abstinence period was recorded. The semen volume was estimated by weighing (1g=1ml), sperm density was identified utilizing Bürker-Türkmecytometer, and total count was measured by multiplication. Sperm morphology was evaluated on Papanicolaou-stained smears according to strict criteria (10). For sperm motility assessment, well-mixed semen placed on a glass slide and examined microscopically at x100 magnification and categorized into progressive motility, non-progressive motility or immotile. The mean of two semen samples was calculated for semen volume, total sperm count, concentration, motility, and morphology.

Fasting blood sampling was taken exclusively between 8.00 and 10.00 a.m. on the same day when the first semen specimen was obtained. The blood sample was analyzed for measurement of total testosterone (TT), E2, FSH, LH, SHBG, vitamin D3 level, and ionized calcium level. Serum FSH, LH was determined by sandwich chemiluminescence immunoassay using (Maglumi 800, Snipe Diagnostic, China). Serum testosterone, E2, SHBG, prolactin, and vitamin D levels were determined by competitive chemiluminescence immunoassay using (Maglumi 800, Snipe Diagnostic, China) while serum ionized calcium was determined using (Cobas 8000, Roche, Switzerland). The ethical committee of our institute approved this study.

Statistical analysis was carried out using Microsoft excel 2016 and statistical package for social sciences version 23 to calculate means, standard deviation, and percentages.

**Results**

The baseline characteristics, serum vitamin D, serum ionized calcium, seminal fluid analysis and reproductive hormones levels of 105 men (52 infertile, 50 fertile) included in the current study were summarized in Table 1.

**Table (1): Characteristics of the studied men.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>InfertilityN=52Mean ± SD</th>
<th>ControlN=50Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(year)</td>
<td>28.12 ± 4.42</td>
<td>29.62 ± 5.55</td>
<td>0.134</td>
</tr>
<tr>
<td>Height(cm)</td>
<td>176.23 ± 8.92</td>
<td>178.29 ± 8.54</td>
<td>0.304</td>
</tr>
<tr>
<td>Weight(kg)</td>
<td>86.69 ± 12.08</td>
<td>83.92 ± 13.906</td>
<td>0.285</td>
</tr>
<tr>
<td>BMI(kg/M²)</td>
<td>24.68 ± 4.19</td>
<td>23.35 ± 3.99</td>
<td>0.104</td>
</tr>
<tr>
<td>Testicular size(ml)**</td>
<td>19.89 ± 1.62</td>
<td>20.43 ± 1.66</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin D(nmol/L)</td>
<td>24.85 ± 17.46</td>
<td>55.88 ± 10.14</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>
S. Ca²⁺(mg/dl)    4.88 ± 0.28    4.8 ± 0.19   0.099
Ejaculate volume(ml)    1.75 ± 0.64    1.94 ± 0.5   0.09
Sperm concentration (million/ml)  35.48 ± 19.7    41.6 ± 22.08   0.142
Sperm morphology (%)    56.69 ± 24.27    62.3 ± 17.26   0.182
Total motility (PR+NP %)  56.63±9.27    79.4 ± 14.55  0.0001
Progressive motile (%)    13.07±11.25    36.8 ± 15.64  0.0001
Non progressive motile (%) 43.75±8.13    42.6 ± 10.81  0.544
Immobile sperms(%)   43.37±7.99    20.2 ± 16.09  0.0001
S.LH(U/L)     8.97 ± 4.47    10.07 ± 3.36   0.164
S.FSH(U/L)  11.34 ± 3.35    7.43 ± 4.05   <0.001*
S.TT(ng/ml)    3.26 ± 2.32    6.84 ± 1.94   <0.001*
S.E2(nmol/l)  55.21 ± 10.5    35.36 ± 9.05 <0.001*
SHBG(nmol/L)  19.08 ± 15.84    35.86 ± 10.1 <0.001*

PR=progressive motility, NP=non progressive motility, LH=luteinizing hormone, FSH=follicular stimulation hormone, E2=estradiol, SHBG=sex hormone binding globulin.

*P value<0.05 significant.**Testicular size measured by u/s

Vitamin D serum level in the infertility group (24.85nmol/L) was very much reduced in comparison to the control group (55.88nmol/L) with significant difference (P <0.001) while serum Ca+2 levels were comparable in both groups (4.8mg/dl) Table 1. Men in the infertility group had lower serum levels of LH, TT, SHBG and higher serum levels of FSH and E2 than men in the control group with significant difference (P value <0.001) between the two groups in all reproductive hormones except serum LH levels (Table 1). Among the 50 males in control group, serum vitamin D levels showed no significant correlation to seminal fluid volume, sperm concentration, and morphology but regarding sperm motility, high levels of serum vitamin D were significantly negatively coordinated with nonprogressive motility (r=-0.318, P= 0.006) and non-motile sperms with (r= -0.478, P= 0.0004) (Table 2).

In the infertile patients group, serum vitamin D levels also showed no significant correlation to seminal fluid volume, sperm concentration, and morphology but the low levels of serum vitamin D among the infertile patients group had significant positive correlation with total motility(r=0.280, P=0.044) and progressive motility (r= 0.435, P= 0.001) also it is significantly negatively correlated with nonprogressive motility (r= - 0.415, P= 0.002). Immotile sperms had no significant correlation with vitamin D levels (Table 2).

Table (2): Relationship between semen parameters and vitamin D in both groups.

Among the fertile male group (control group) serum vitamin D levels showed no statistically significant correlation with serum LH, FSH, TT, E2 hormones levels and sex hormone binding globulin, however low serum vitamin D levels were associated with low serum TT and SHBG levels among the infertile male patients group with significant positive relation only with SHBG (r= 0.364 and P= 0.017). Although the low serum vitamin D levels were associated with high FSH, LH and E2 hormonal levels, no statistically significant correlation was found with the latter variables (Table 3).

Table (3): Correlation between vitamin D and reproductive hormones in both groups.

<table>
<thead>
<tr>
<th>Vitamin D</th>
<th>Semen volume</th>
<th>Sperm concentration</th>
<th>Sperm morphology</th>
<th>Total motility</th>
<th>Progressive motility</th>
<th>Non progressive motility</th>
<th>immotile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertility</td>
<td>p 0.543</td>
<td>0.906</td>
<td>0.397</td>
<td>0.044*</td>
<td>0.001*</td>
<td>0.002*</td>
<td>0.117</td>
</tr>
<tr>
<td>r 0.086</td>
<td>-0.016</td>
<td>0.012</td>
<td>0.280</td>
<td>0.435</td>
<td>-0.415</td>
<td>-0.220</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>p 0.051</td>
<td>0.869</td>
<td>0.568</td>
<td>0.085</td>
<td>0.656</td>
<td>0.006*</td>
<td>0.0004*</td>
</tr>
<tr>
<td>r 0.278</td>
<td>-0.024</td>
<td>0.082</td>
<td>0.246</td>
<td>-0.06</td>
<td>-0.318</td>
<td>-0.478</td>
<td></td>
</tr>
</tbody>
</table>

*P value<0.05 significant. r: correlation coefficient.

Among the 50 males included in the control group, serum Ca+2 levels showed no statistically significant correlation to seminal fluid volume, sperm concentration, sperm morphology, and sperm motility (Table 4). However in the infertile patients group despite serum Ca2+ levels showed no statistically significant correlation to seminal fluid volume, sperm concentration and morphology, low levels of serum Ca+2 were significantly positively correlated with total sperm motility, progressive motility and it was negatively correlated to immotile sperm, but no statistically significant correlation with non-progressive motile sperms (Table 4).

Table (4): Correlation between ionized calcium levels and seminal parameters in both groups.

<table>
<thead>
<tr>
<th>Vitamin D</th>
<th>Semen volume</th>
<th>Sperm Concentration</th>
<th>Sperm morphology</th>
<th>Total motility</th>
<th>Progressive motility</th>
<th>Non progressive motility</th>
<th>immotile</th>
</tr>
</thead>
</table>

Among the fertile males control group, serum Ca+2 levels showed no statistically significant correlation to serum LH, FSH, TT, E2 hormones levels and SHBG (Table 5). On the contrary, in the infertile group, low serum Ca+2 levels were associated significantly with low LH and FSH hormonal levels with significant positive correlation ($r= 0.437$ and $P= 0.001$, $r= 0.311$ and $P= 0.029$ respectively). Although low serum Ca+2 levels were associated with high E2 hormonal levels, no statistically significant correlation was found ($r= -0.200$ and $P= 0.052$). Also, both serum TT hormone and SHBG levels showed no significant correlation to the serum Ca+2 levels in the current study (Table 5).

**Table (5):** Correlation between ionized calcium levels and reproductive hormones in both groups.

<table>
<thead>
<tr>
<th></th>
<th>LH</th>
<th>FSH</th>
<th>TT</th>
<th>E2</th>
<th>SHBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertility</td>
<td>P: 0.001*</td>
<td>0.029*</td>
<td>0.096</td>
<td>0.052</td>
<td>0.071</td>
</tr>
<tr>
<td></td>
<td>r: 0.437</td>
<td>0.311</td>
<td>0.233</td>
<td>-0.200</td>
<td>0.252</td>
</tr>
<tr>
<td>Control</td>
<td>P: 0.332</td>
<td>0.054</td>
<td>0.966</td>
<td>0.316</td>
<td>0.242</td>
</tr>
<tr>
<td></td>
<td>r: 0.140</td>
<td>0.274</td>
<td>0.006</td>
<td>-0.144</td>
<td>-0.168</td>
</tr>
</tbody>
</table>

**Discussion**

The existence of a VDR in different portions of the menu system is potentially linked to numerous activities of the reproductive axis in human being, VDR and metabolizing enzymes are demonstrated in human sperms. Therefore, it appears that vitamin D has a direct effect on semen quality (11). Our findings showed significantly reduced vitamin D levels amongst infertile men contrasted to the controls, this finding was consistent with other studies (AL_Baldawyi et al, (12), Hassan et al, (13), Wadhwa et al, (14), Abbasihormozi et al, (11) and Arab et al, (15) which reported a deficient level of serum vitamin D in the infertile men. In other studies conducted, in infertile men the mean level of vitamin D was insufficient (25-50 nmol/L) (Hammoud et al,(16) and in the sufficient level (50-75nmol/L) (Jensen et al, (17)), the difference in socioeconomic condition, skin pigmentation and sample size can explain the difference in mean vitamin D levels among these studies.

Calcium is needed to commence the acrosome reaction liberating enzymes necessary for effective sperm-ovum communication (18), and vitamin D through activation of VDR raised the intracellular calcium from its store in the sperm’s neck stimulating sperm motility and acrosome reaction (19), therefore calcium action on male fertility may be related to the action of vitamin D, so in this study, we evaluated the serum level of Ca+2 in the infertile men in addition to serum vitamin D, the infertile male group had a serum level of Ca+2 similar to the fertile men.
with mean of (4.88mg/dl) without significant difference this was in accordance with (Abbasihormozi et al, (11) and Jensen et al, (8, 17) who reported a comparable serum Ca+2 in fertile and infertile men. On the contrary, this finding disagreed with Bassey et al, (20) who reported a significant low serum Ca+2 in infertile men, the difference in the methods of measurements in the laboratories could be attributed to this differences.

In this study the infertile men had a normal seminal fluid parameters except low progressive motility, this was in accordance with other studies presented by Hadwin et al, (21) and Abu Raghif (22) and indifference with Jensen et al, (17) which reported lower sperm count, concentration and morphology, this could be due to the difference in studies inclusion criteria as they included all infertile men and some of them had other urogenital pathologies like varicocele and cryptorchidism which might affect seminal fluid analysis quality.

The infertile group in the current study characterized by higher serum levels of FSH with significant difference in comparison with the control group, this was inconsistent with Mahdi et al, (23) and Safarinejad et al, (24) but it was opposing to the findings of another research by Abed et al, (25) in which no significant difference was reported. FSH shows the vital function in creating and maintaining spermatogenesis. The measurement of serum FSH is a valuable index for the testicular histological status. A raised level of FSH in men may point to abnormalities in initial sperm production (23). LH level in the infertile male in our study was lower than that of the control group but it was not significant, this was similar to Mahdi et al, (23) and Yenzeel (26) but disagreed with Abed et al, (25) which showed a significantly low level of LH in their infertile men.

Serum TT was significantly low in our infertile group when compared with the fertile men, This finding seemed to be in line with Mahdi et al, (23), Yenzeel (26) and also by Andersson et al, (27) showing evidence of Leydig cell dysfunction in infertile men leading to impairment in testosterone production. In contrast to our observation Safarinejad et al, (24) had found that infertile men have TT levels comparable to healthy men, However, this study was generally based on a small study group that might be too small to reach valid conclusions.

High serum level of E2 found in infertile male of the present study was in accordance with Andersson et al, (27) but in conflict with Gregoriou et al, (28), as this study showed lower serum E2 levels in the infertile men, the difference in the inclusion criteria could explain the variation since they included infertile men only with low testosterone/estradiol ratio. Men with abnormal sperm parameters appear to commonly have excess aromatase activity which converts testosterone to E2 (29) and leads to high serum E2 level. Change of plasma E2 levels may be linked with significant alteration in plasma LH levels via an influence at the pituitary gland level (negative feedback) (28) which in turn affects the release of testosterone. Testosterone hormone is very essential male hormone stimulated by LH which induce Leydig cells to liberate testosterone from the testis (23), therefore the high E2 level in our infertile male group might explain the low LH and testosterone levels by negative feedback action on the anterior pituitary. SHBG was significantly low in our infertile men group this was consistent with the previous studies reported by Safarinejad et al, (30, 31) while Andersson et al, (27) stated no difference between
infertile and fertile men. SHBG levels differ excessively among individuals and rely on agents like diet, BMI, insulin levels, age, and thyroid function and could attribute to this difference. A study in a large number of neonates and men across a wide age range confirms that levels of SHBG can vary widely among individuals (32). Keeping in mind laboratory bias and different methods of blood sample analysis could also lead to the divergences in the hormonal assessment.

The present study proved the effect of vitamin D levels on sperm motility. Low levels of vitamin D associated with low total motility and low progressive motility, The relation linking vitamin D with sperm motility was in consistence with data from other studies who had examined this relation and our finding was closely in accordance with (Jensen et al, (17)(19), Tirabassi et al, (33), Arab et al,(15) and Abbasihormoziet al, (11)), this finding was also supported by the finding of VDR and vitamin D metabolizing enzyme in the head of the sperms which was reported by Jensen et al, (34), in the same line in vitro studies displayed the positive effect of vitamin D on human sperms (35) and by Wadhwaet al, (14) who reported improvement in sperm motility following vitamin D supplementation. However, in this regard, contrary finding was presented reported by Hassan et al, (13) who described no association linking vitamin D and sperm motility and Ramlau-Hansen et al, (36) who stated that high levels of vitamin D were associated with poor seminal fluid parameters. Regarding other sperm characteristics (count, concentration, morphology, and volume) no significance with serum vitamin D levels was identified in our study, this was in accordance with Arab et al, (15), Abbasihormoziet al, (11) and in contrast with Ramlau-Hansen et al, (36), the presence of this heterogeneity between studies could be due to the fact that some of the studies with different finding were conducted on normal fertile men. Furthermore, there were some other factors that may be related to semen quality parameters including age, BMI and lifestyle. The effect of vitamin D on male reproduction is argued process. Ideal sperm action is influenced directly through the impact of vitamin D or indirectly by calcium homeostasis (11).

Lack of the VDR-controlled calcium transporter, who found in the epididymis, creates diminished cellular calcium transference with variations in epididymal fluid density and the resulting deterioration in spermatozoa motility and infertility (13). This was in the same line with our finding that serum Ca+2 positively correlated with total motility and progressive motility and also in accordance with Marin-Briggler et al(37) and Hamadet al, (18) who demonstrated that low levels of serum Ca+2 among infertile men was associated with sperm hypomotility and Jensen et al,(19) who mentioned that increase in intracellular Ca+2 in human spermatozoa might induce sperm motility, these showed the value of calcium in sperm functioning, comprising motility. However other study performed by Jensen et al, (17) showed that high levels of Ca+2 linked with low sperm motility. Except for motility other sperm parameters in the current study had no significant correlation with Ca+2, this was in accordance with Colagar et al,(38) where they established that serum Ca+2 did not correlate with sperm quality. Hamad et al,(39) demonstrated that sperm volume, morphology in addition to sperm motility differ significantly between normal and abnormal seminal calcium levels, the discrepancies among these studies might be attributed to the assessment of seminal calcium or total serum calcium instead of serum ionized calcium.
In our study, there wasn’t any significant association between reproductive hormones (TT, LH, FSH, E2) and vitamin D, the findings presented by Wadhwa et al., Hammoud et al., and Abbasihormozi et al. were in accordance with our findings, other study accomplished by Jensen et al. had the same results except they demonstrated a significant negative correlation with E2, our study also had negative correlation between vitamin D and E2 but it was not a significant finding, the small number of population participated in this study might explain this insignificance. Ramlau-Hansen et al. revealed similar outcomes excepting a positive correlation with FSH, this was because their study included a healthy male in contrast to ours which included infertile men. Chin et al. reported a significant positive correlation with TT, this study done among Malaysian men and the results might not be representative of the whole. Considering all these findings, our results did not prove a stimulatory influence of vitamin D on the product of testicular testosterone and anterior pituitary production of LH and FSH.

We demonstrated a significant positive relationship between SHBG and vitamin D where low levels of vitamin D associated with low SHBG levels. Ramlau-Hansen et al., Jensen et al., Chin et al., and Wehr et al. reported the same finding. Nevertheless, Hammoud et al. showed no difference in SHBG with vitamin D deficiency. SHBG is a serum-steroid transporting protein, it is mainly synthesized in the liver. SHBG is altered in a variety of medical conditions and states like thyroid function, liver function, obesity and age that could contribute to variances among studies.

In the current study serum ionized Ca+2 correlated significantly with the gonadotropins (LH and FSH), in this aspect a study by Thompson et al. concluded that rapid gonadotropin secretion and activation have been attributed to gonadotropin-releasing hormone (GnRH) dependent on calcium mobilization, Loss et al. described that the rapid signaling actions of FSH in mature Sertoli cells mainly related to Ca2+ inflow and the electrophysiological changes. This evidence suggests that calcium might have an action on the hypothalamic-pituitary-testicular axis. Serum TT, E2, SHBG didn’t show significant correlation with serum Ca+2 in our study, Salem et al. described the same finding. Jensen et al. demonstrated no correlation with serum TT, SHBG but a negative correlation with E2 was concluded, our study also had a negative correlation but with a borderline P value (0.052), differences in the sample size, methods used in the measurement by the laboratories and commercially available kits used could be the reason for these dissimilarities. In Conclusion, serum vitamin D and Ca+2 revealed a significant correlation with sperm motility reflecting their important function in male infertility as well as determining that both are significantly linked to the seminal fluid quality and some reproductive hormones in infertile men.

Conflict of interest: None

Funding sources: None

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19. Jensen MB, Bjerrum PJ, Jessen TE, et al. Vitamin D is positively associated with sperm motility and


