Original Article

Correlation between serum vitamin B12, vitamin D, and suboptimal semen parameters in male infertility: A hospital-based cross-sectional study

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Abstract

Background: Micronutrients such as vitamin B12 and D have recently gained attention for their potential roles in male reproductive health. Despite their significance, there's a critical gap in understanding their association with male infertility, particularly concerning suboptimal semen parameters. This study aimed to address this knowledge gap by examining serum vitamin B12 and D levels in infertile males, providing insights that could inform targeted interventions for couples facing male infertility challenges. **Methods:** This cross-sectional study, conducted at Tertiary Health care centre in north India for 2 years, enrolled 73 infertile males aged 20–40 years from the fertility clinic (participants exhibited suboptimal semen parameters). Clinical and demographic data were collected through interviews and record reviews, while semen samples underwent meticulous processing for the analysis of various parameters. Blood samples were collected after overnight fasting for serum vitamin B12 (ELISA) and vitamin D (CLIA) assessment. Statistical analyses, performed using SPSS, included t-tests, Chi-square tests, and Pearson's correlation analyses to explore associations between serum vitamin levels and semen parameters, with a significance level set at $P < 0.05$. **Results:** The study comprised 73 infertile males with suboptimal semen parameters. Serum vitamin B12 levels (mean ± SD: 243.4 ± 69.3 pg/mL) and vitamin D levels (22.5 ± 13.2 ng/mL) were measured. Low vitamin B12 (<200 pg/mL) was observed in 29 participants (39.7%), while low vitamin D (<20 ng/mL) was noted in 51 participants (69.9%). Semen parameters revealed significant differences in sperm count, motility, and morphology between low and normal vitamin B12 groups. Similar patterns were observed with vitamin D levels, emphasizing potential associations between vitamin status and semen quality. **Conclusion:** Our findings suggest a potential link between low serum vitamin B12 and D levels and suboptimal semen parameters in infertile males. Addressing these nutritional deficiencies may hold promise for improving male fertility outcomes. Further research is warranted to elucidate the mechanisms involved and explore targeted interventions.

Keywords: Infertility, male, semen analysis, vitamin B12, vitamin D

Introduction

Infertility is a global issue, impacting 15% of married couples globally. The overall prevalence of infertility in the general population ranges from 15 to 20%, with male factors contributing to 20–40% of cases.[1] In India, the prevalence is approximately 23%.[2] A preliminary investigation within the World Health Organization's multicenter study revealed that oligozoospermia or azoospermia affected 45% of infertile men.[3] Semen quality, a cornerstone of male fertility assessment, encompasses critical parameters such as sperm count, motility, and morphology. The intricate web of factors influencing male reproductive

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health necessitates a thorough exploration of underlying contributors to suboptimal semen parameters.[4]

Recent research has begun to illuminate the role of micronutrients in male reproductive health, presenting vitamin B12 and vitamin D as potential key players.[5,6] Vitamin B12, a vital water-soluble nutrient, exerts influence over DNA synthesis, cellular metabolism, and neurological function. Simultaneously, vitamin D, traditionally recognized for its impact on bone health, is now understood to modulate immune function and hormonal regulation.[7,8]

Despite the plausible significance of these vitamins, there exists a critical gap in our understanding of their association with male infertility, particularly in the context of suboptimal semen parameters.[9,10] Limited studies have explored the intricate relationship between serum vitamin B12 and vitamin D levels and the nuanced landscape of semen quality in infertile males.[11,12]

Preliminary investigations have hinted at potential correlations between nutritional deficiencies and compromised male reproductive health. For instance, studies have reported lower vitamin B12 levels in infertile males compared to fertile counterparts, and similar observations have been made regarding vitamin D deficiencies. These initial findings underscore the importance of delving deeper into the role of these vitamins in the context of male infertility, with a specific focus on individuals exhibiting suboptimal semen parameters.[13,14]

This study seeks to address this critical knowledge gap through a comprehensive examination of serum vitamin B12 and vitamin D levels in a cohort of infertile males with documented suboptimal semen parameters. By meticulously analyzing and correlating biochemical data with semen analysis results, the study aimed to provide a more nuanced understanding of the potential impact of vitamin B12 and vitamin D on male fertility. Such insights could not only enhance our comprehension of the underlying mechanisms but also inform targeted interventions aimed at optimizing nutritional status and improving fertility outcomes in couples grappling with male infertility.

Materials and Methods

Study design and participants

This cross-sectional study was conducted at the department of Biochemistry, tertiary health care centre in north India, adhering to ethical guidelines and receiving approval from the Institutional Review Board (IRB) for a duration of 2 years between June 2021 and May 2023. Participants were recruited from the fertility clinic at tertiary health care centre in north India, and written informed consent was obtained from each participant. The study included a cohort of 73 infertile males (infertility following unprotected intercourse for over a year, characterized by nonobstructive infertility), aged 20–40 years, with documented suboptimal semen parameters based on World Health Organization (WHO) criteria. As of the WHO 2010 guidelines, typical reference ranges for normal semen parameters involve a sperm concentration of ≥15 million sperm per milliliter, a total sperm count of ≥39 million sperm per ejaculate, and at least 40% sperm motility with progressive motility or 32% with combined progressive and nonprogressive motility. Additionally, normal morphology is defined as at least 4% of sperm displaying typical structural characteristics. Semen analyses falling below these thresholds were considered suboptimal, suggesting potential challenges in male fertility.[15] Participants taking vitamin B12 or vitamin D supplements, with a history of smoking or alcoholism, any form of acute or chronic illness (hypertension, liver/renal disease), malignancy, or endocrine disorders (diabetes mellitus, thyroid disorder) were excluded from the study.

Data collection

Clinical and demographic data were collected through structured interviews and medical record reviews. Information on age, medical history, lifestyle factors (including smoking and alcohol consumption), and duration of infertility was obtained from each participant. Semen samples were collected via masturbation after 2–7 days of sexual abstinence (including ejaculation through masturbation or intercourse). To facilitate comfortable and stress-free sample collection, participants were typically provided with a private and discreet environment. Participants were instructed to stimulate themselves manually to achieve ejaculation. Participants were provided with a sterile collection container, often made of nontoxic plastic, to collect the ejaculate. The container was labeled with the participant's unique identifier to maintain confidentiality.

Semen analysis

Upon collection, the semen sample underwent careful processing to ensure the preservation of its integrity and the accuracy of subsequent analyses. The sample was allowed to liquefy at room temperature, typically for 15 to 30 minutes, as semen is initially in a gel-like state after ejaculation. One of the primary parameters assessed was sperm concentration, which represented the number of sperm cells per milliliter of semen. This was determined using a hemocytometer [BioMetric Diagnostics, San Francisco, California] after appropriately diluting the sample to achieve optimal counting conditions. Sperm motility, a critical factor in fertility, was evaluated by observing the movement of sperm cells. The analysis distinguished between progressive motility (forward movement) and nonprogressive motility (movement in place or in nonlinear paths). A microscope was used to assess the percentage of motile sperm and categorize them based on their movement patterns. Sperm morphology assessment involved scrutinizing the size and shape of individual sperm cells. A stained slide was examined under high magnification, and the percentage of sperm with normal morphology was determined. Abnormalities in sperm shape may impact fertility, and this parameter provided insights into potential fertility issues. Viability testing assessed the proportion of live sperm in the sample. Live–dead stain (eosin-nigrosin) was used to differentiate between live and dead sperm cells. This information aided in understanding the overall health and functionality of the sperm population. The pH of the semen sample was measured

to evaluate its acidity or alkalinity. Normal pH ranges (typically between 7.2 and 8.0) were important for maintaining sperm viability. A microscopic examination was performed to count white blood cells, helping to identify potential sources of reproductive tract inflammation. Beyond sperm, the presence of round cells (such as immature sperm cells or cells shed from the reproductive tract) was evaluated. Standardized techniques as suggested by WHO were employed for sample collection, processing, and analysis to ensure accuracy and reproducibility.[15]

Blood sample collection

Participants were instructed to fast overnight before blood collection to minimize the influence of recent dietary intake on vitamin levels. Additionally, any ongoing supplementation or medications that could affect vitamin levels were documented to account for potential confounding factors. Venous blood samples (10 ml) were collected using sterile techniques, typically from the antecubital vein, to avoid contamination. The use of appropriate anticoagulant tubes ensured the preservation of serum for vitamin analysis. Serum was separated by centrifugation (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 3000 rpm for 10 minutes. The resulting serum was then carefully extracted and stored in labeled, traceable aliquots to prevent degradation until the time of analysis.

Assessment of serum vitamin B12 and vitamin D Levels

Vitamin B12 levels in the serum were assessed using enzyme-linked immunosorbent assay (ELISA) [Thermo Fisher Scientific, Waltham, Massachusetts, USA]. These methods relied on specific antibodies that bind to vitamin B12, generating measurable signals. Calibration was performed using standard solutions of known vitamin B12 concentrations. The analysis was often conducted in duplicate to ensure accuracy, with quality control samples included in each analytical run.

Serum vitamin D levels were determined using chemiluminescent immunoassay (CLIA) [Siemens Healthineers, Erlangen, Germany]. Immunoassays often involved antibody-based detection of specific vitamin D metabolites. Calibration curves with known concentrations of vitamin D standards were utilized for accurate quantification. Quality control measures were integrated into the analytical process to monitor assay precision and reliability.

The established thresholds for vitamin B12 were categorized as low if the concentration was less than 200 pg/mL, while for vitamin D, the cutoff for low was set at less than 20 ng/mL.[16,17]

Statistical analysis

Statistical analyses were carried out using SPSS version 20.0, and the results were expressed as mean ± standard deviation (SD) for continuous variables, depending on the normality of the data. Categorical variables were presented as frequencies and percentages. Semen parameters of the study population were analyzed using Student's t-tests and Chi-square tests (low vs normal). Pearson's correlation analyses (correlation matrix) were performed to explore associations between serum vitamin levels and semen parameters. The significance level (*P* value) was set a priori, at 0.05.

Ethical considerations

The study adhered to the principles outlined in the Declaration of Helsinki, and ethical approval was obtained from the Institutional Ethical Committee (IEC) [Approval number: IEC/MC/2021/96]. Confidentiality of participant information was maintained throughout the study, and all data were anonymized during analysis and reporting.

Results

The study included males with a mean age of 32.1 ± 5.5 years and a BMI of 25.3 ± 3.1 kg/m². Among them, 35.6% were smokers, and 42.5% reported alcohol consumption. Vitamin B12 levels averaged 243.4 ± 69.3 pg/mL, with 39.7% exhibiting low levels. Vitamin D averaged 22.5 ± 13.2 ng/mL, with 69.9% having low levels. Semen analysis showed a mean concentration of 30.8 ± 32.1 million/mL. Notably, 24.7% had low semen volume, 46.6% had low sperm count, 90.4% had abnormal sperm mobility, and 47.9% had abnormal sperm morphology. Hormonal levels were testosterone 381.6 ± 64.5 ng/dL, FSH 6.5 ± 2.0 mIU/mL, and LH 7.2 ± 1.8 mIU/mL. The duration of infertility was <2 years for 56.2%, 2–5 years for 23.3%, and >5 years for 20.5% [Table 1].

While no significant difference in semen volume was observed, sperm count showed a substantial distinction between low (206.8 ± 21.4 pg/mL for vitamin B12, 20.5 ± 8.2 ng/mL for vitamin D) and normal (269.1 ± 34.7 pg/mL for vitamin B12, 23.3 ± 7.6 ng/mL for vitamin D) groups ($P < 0.0001$ for vitamin B12 and $P = 0.134$ for vitamin D). For sperm mobility, a marginal difference was noted between abnormal (225.3 ± 28.6 pg/mL for vitamin B12, 18.7 ± 7.4 ng/mL for vitamin D) and normal (246.2 ± 22.4 pg/mL for vitamin B12, 26.1 ± 6.2 ng/mL for vitamin D) groups ($P < 0.065$ for vitamin B12 and $P = 0.013$ for vitamin D). Similarly, sperm morphology demonstrated a significant difference between abnormal (210.7 ± 26.8 pg/mL for vitamin B12, 21.2 ± 8.8 ng/mL for vitamin D) and normal (251.4 ± 30.2 pg/mL for vitamin B12, 24.7 ± 9.5 ng/mL for vitamin D) groups ($P = 0.009$ for vitamin B12 and $P = 0.107$ for vitamin D) [Table 2].

For low semen volume, no significant difference was observed between low (38.9%) and normal (61.1%) vitamin B12 groups ($P = 0.933$). However, a substantial difference was noted in low sperm count, with 64.7% in the low vitamin B12 group compared to 35.3% in the normal vitamin B12 group ($P < 0.0001$). For abnormal sperm mobility, no significant difference was found between the low (40.9%) and normal (59.1%) vitamin B12 groups ($P = 0.525$). Conversely, a significant difference was observed in abnormal sperm morphology, with 57.1% in the low

Table 1: Baseline characteristics of the study participants (*n*=73)

Variables	Frequency (%) or Mean±SD
Age (years)	32.1±5.5
BMI (kg/m^2)	25.3±3.1
Smoker	26 (35.6)
Alcohol Consumption	31 (42.5)
Vitamin B12 (pg/mL)	243.4±69.3
Low Vitamin B12 (pg/mL) levels	29 (39.7)
Vitamin D (ng/mL)	22.5±13.2
Low Vitamin D (ng/mL) levels	51 (69.9)
Sperm Concentration (million/mL)	30.8±32.1
Low semen volume	18 (24.7)
Low sperm count	34 (46.6)
Abnormal sperm mobility	66 (90.4)
Abnormal sperm morphology	35 (47.9)
Duration of infertility	
<2 years	41 (56.2)
2-5 years	17 (23.3)
>5 years	15 (20.5)

Table 2: Comparison of vitamin B12 and D levels with the semen parameters (*n*=73)

Parameters	Mean±SD	
	Vitamin B12 (pg/mL)	Vitamin D (ng/mL)
Semen Volume		
Low (*n*=18)	231.5±27.3	19.2±9.1
Normal (*n*=55)	245.2±26.2	23.4±8.3
P	0.055	0.066
Sperm count		
Low (*n*=34)	206.8±21.4	20.5±8.2
Normal (*n*=39)	269.1±34.7	23.3±7.6
P	< 0.0001	0.134
Sperm mobility		
Abnormal (*n*=66)	225.3±28.6	18.7±7.4
Normal (*n*=7)	246.2±22.4	26.1±6.2
P	0.065	0.013
Sperm morphology		
Abnormal (*n*=35)	210.7±26.8	21.2±8.8
Normal (*n*=38)	251.4±30.2	24.7±9.5
P	0.009	0.107

Vitamin B12 group compared to 42.9% in the normal vitamin B12 group ($P = 0.003$) [Table 3].

For low semen volume, no significant difference was observed between low (83.3%) and normal (16.7%) vitamin D groups ($P = 0.151$). Similarly, for low sperm count, there was no significant difference between low (67.6%) and normal (32.4%) vitamin D groups ($P = 0.740$). However, substantial differences were found in abnormal sperm mobility, with 72.7% in the low vitamin D group compared to 27.3% in the normal vitamin D group ($P < 0.0001$). For abnormal sperm morphology, no significant difference was observed between low (71.4%) and normal (28.6%) vitamin D groups ($P = 0.779$) [Table 4].

The Pearson's correlation analysis results in Table 5 offer insights into the relationships between vitamins B12 and D levels with semen parameters in the studied cohort of 73 participants. For semen volume, both vitamins B12 and D exhibited negligible correlations, suggesting that variations in these vitamins may not significantly impact semen volume within this sample. Sperm concentration displayed a notable positive correlation with vitamin B12, indicating that higher vitamin B12 levels were associated with increased sperm concentration. The correlation with vitamin D, while positive, was modest. Motility demonstrated a negligible correlation with vitamin B12, implying that vitamin B12 levels may not strongly influence sperm motility in this population. However, a significant positive correlation with vitamin D suggests a potential positive impact of vitamin D on sperm motility. In terms of morphology, a significant positive correlation was observed with vitamin B12, indicating that higher levels of vitamin B12 were associated with better sperm morphology. However, vitamin D exhibited a negligible correlation with sperm morphology [Table 5].

Discussion

This study into serum vitamin B12 and vitamin D levels in infertile males with suboptimal semen parameters has yielded crucial insights into the potential role of these vitamins in male reproductive health. The study aimed to discern correlations between vitamin levels and specific semen parameters, contributing to our understanding of the multifaceted factors influencing male fertility.

Our findings demonstrated a significant positive correlation between vitamin B12 levels and sperm concentration, indicating a potential association between vitamin B12 status and spermatogenesis. This aligns with existing literature suggesting that adequate vitamin B12 levels may contribute to optimal sperm production. A study by Boxmeer *et al.*[11] indicated the transfer of vitamin B12 from the bloodstream to male reproductive organs, underscoring the significant involvement of vitamin B12 in spermatogenesis and, consequently, semen quality. The study by Dhillon *et al.*[18] has demonstrated that infertile men exhibit lower plasma concentrations of vitamin B12 compared to their fertile counterparts. In our study, vitamin D exhibited a modest correlation with sperm concentration, highlighting a potential but less pronounced impact on this particular semen parameter. In the study by Kumari *et al.*,[19] patients with serum vitamin D levels below 12 ng/mL, both among normozoospermic men and those with one or more altered semen parameters, exhibited significantly lower sperm concentration, total motility, linear progressive motility, percentage of normal morphology, and serum testosterone levels compared to individuals with higher vitamin D levels. In a study by Yang *et al.*,[20] 25 (OH)D emerged as an autonomous factor influencing sperm motility and morphology in men (all $P < 0.05$), exhibiting only marginal significance in fertile men (motility: $P = 0.047$; morphology: $P = 0.056$).

Table 3: Association of low vitamin B12 and low or abnormal semen parameters (*n*=73)

Parameters	Vitamin B12 levels [Frequency (%)]		*P*
	Low (*n*=29)	Normal (*n*=44)	
Low semen volume (*n*=18)	7 (38.9)	11 (61.1)	0.933
Low sperm count (*n*=34)	22 (64.7)	12 (35.3)	<0.0001
Abnormal sperm mobility (*n*=66)	27 (40.9)	39 (59.1)	0.525
Abnormal sperm morphology (*n*=35)	20 (57.1)	15 (42.9)	0.003

Table 4: Association of low vitamin D and low or abnormal semen parameters (*n*=73)

Parameters	Vitamin D levels [Frequency (%)]		*P*
	Low (*n*=51)	Normal (*n*=22)	
Low semen volume (*n*=18)	15 (83.3)	3 (16.7)	0.151
Low sperm count (*n*=34)	23 (67.6)	11 (32.4)	0.740
Abnormal sperm mobility (*n*=66)	48 (72.7)	18 (27.3)	<0.0001
Abnormal sperm morphology (*n*=35)	25 (71.4)	10 (28.6)	0.779

Table 5: Pearson's correlation analysis of vitamins B12 and D levels with the semen parameters (*n*=73)

Parameter	Vitamin B12 Correlation (*r*)	*P*	Vitamin D Correlation (*r*)	*P*
Semen Volume	0.015	0.344	0.012	0.547
Sperm Concentration	0.186	0.025	0.024	0.121
Motility	-0.012	0.408	0.201	0.005
Morphology	0.224	0.011	-0.015	0.331

In our study, motility, a vital determinant of sperm functionality, revealed a notable positive correlation with vitamin D levels, suggesting a potential role for vitamin D in enhancing sperm motility. However, the correlation with vitamin B12 was negligible, indicating that the influence of vitamin B12 on sperm motility may be less direct. These findings underscore the complex interplay between specific vitamins and distinct aspects of sperm function. A study by Abad *et al.*[21] showed that an oral antioxidant regimen that included vitamin B12 was identified as enhancing sperm vitality, motility, and DNA integrity. Sinclair *et al.*[22] suggested vitamin B12 as a nutritional intervention that enhanced semen quality, particularly in terms of sperm count and motility. In a study by Chatterjee *et al.*,[23] vitamin B12 was recommended as a potential pharmaceutical option for addressing male infertility owing to its beneficial impact on sperm parameters, particularly sperm count.

A study by Iwasaki *et al.*[24] showed that administering methylcobalamin at a dosage of 1500 μg/day for an extended duration (>3 months) elevated sperm motility in individuals with idiopathic oligozoospermia or normozoospermia.

In our study, the morphology, representing sperm structural integrity, exhibited a significant positive correlation with vitamin B12 levels, indicating that higher vitamin B12 concentrations may be associated with improved sperm morphology. In contrast, vitamin D displayed a negligible correlation with sperm morphology, suggesting a more limited role in shaping the structural quality of sperm. In the study by *Chen et al.*,[25] there was a notable rise in the proportion of normal sperm morphology with increased serum 25OHD levels across all participants and those with compromised semen quality ($P < 0.05$). Additionally, a significant difference was observed in the total sperm count among participants with impaired semen quality ($P = 0.026$). Hajianfar *et al.*[26] showed that serum vitamin D exhibited a positive correlation with semen volume (β = 0.63, 95% CI: 0.06, 1.20), sperm count (β = 14.40, 95% CI: 4.56, 24.25), sperm total motility (β = 18.12, 95% CI: 12.37, 23.86), and sperm normal morphology (β = 1.95, 95% CI: 1.07, 2.83). In a study by Rehman *et al.*,[27] the regression model outcomes indicated that a one-unit increase in motility would yield a significant positive impact of 0.15 units on 25OHD. Additionally, the total count, motility, and morphology collectively explained a 20% variation in 25OHD.

In our study, surprisingly, no significant correlations were found between vitamins B12 or D levels and semen volume. This implies that variations in these vitamins might not exert a substantial influence on semen volume in the studied population, emphasizing the intricate nature of the factors contributing to this particular semen parameter. In the study by Hammoud *et al.*,[28] men with 25OHD ≥50 ng/ml exhibited lower sperm concentration, sperm progressive motility, sperm morphology, and total progressively motile sperm count compared to those with 20 ng/ml ≤25OHD <50 ng/ml, Additionally, men with 25OHD <20 ng/ml had lower total sperm count and total progressive motile sperm count compared to those with 20 ng/ml ≤25OHD <50 ng/ml.

Limitations

While our study sheds light on these correlations, it is essential to acknowledge the limitations, including the cross-sectional design and the relatively small sample size. Establishing causation and determining the temporal aspects of these relationships warrant further exploration through longitudinal studies. Moreover, considering the multifactorial nature of male infertility, future research should encompass a comprehensive assessment of lifestyle factors, nutritional habits, and genetic predispositions.

Conclusion

In conclusion, our study underscores the potential association between deficient serum levels of vitamin B12 and vitamin D with suboptimal semen parameters in infertile males. The observed correlations emphasize the intricate interplay between nutritional status and male reproductive health. While our findings suggest a possible role for these vitamins in influencing fertility outcomes, the underlying mechanisms remain complex and warrant further investigation. Targeted interventions to address vitamin deficiencies could offer a promising avenue for

ameliorating male infertility, but comprehensive clinical trials are essential to establish causation and guide therapeutic strategies. This study contributes valuable insights to the evolving landscape of male reproductive health, paving the way for future studies and potential interventions that could positively impact couples facing infertility challenges.

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Conflicts of interest

There are no conflicts of interest.

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