Immunopathologia Persa

DOI:10.34172/ipp.2023.39480

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Does folate deficiency alter sperm parameters in infertile males?

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Received 19 Dec. 2022
Accepted 10 Mar. 2023
Published online 17 May 2023

Keywords: Folate, Sperm parameters, Male infertility

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Abstract

Introduction: There is not enough evidence regarding the influence of folate deficiency in the etiology of idiopathic male infertility.
Objectives: The objective of the current study was to examine whether there is an association between serum levels of folate and sperm parameters among infertile males.
Patients and Methods: This cross-sectional study was undertaken on a sample of infertile males referring to an infertility treatment clinic in Isfahan province, Iran. Semen parameters, comprising sperm concentration, progressive/total motility, morphology, semen leukocyte, agglutination, and vitality were assessed based on standard protocols. The sperm DNA fragmentation assay (SDFA) and sperm chromatin maturity assay (SCMA) were also assessed by the sperm chromatin dispersion and aniline-blue tests respectively.
Results: In general, 70 infertile males including 46 with sufficient serum folate level and 24 with insufficient serum folate level were examined. Subjects with sufficient serum folate level had a significantly higher mean of sperm concentration, vitality, progressive motility, total motility, SDFA, and SCMA ($P<0.05$). Higher serum folate level was also associated with lower risk of abnormal sperm agglutination (OR: 0.09, 95% CI: 0.01-0.65), and morphology (OR: 0.12, 95% CI: (0.02-0.60) after adjustment for potential confounders.
Conclusion: The present study found a significant association between serum folate level and infertility among Iranian infertile males. However, prospective large-scale population-based research is required to confirm the finding.

Citation: Alamdari SS, Derakhshan M, Derakhshan M, Mahzouni P. Does folate deficiency alter sperm parameters in infertile males? Immunopathol Persa. 2023;x(x):e39480. DOI:10.34172/ipp.2023.39480.

Introduction

Infertility has been defined as the failure to conceive after 12 months of regular, unprotected sexual intercourse by the World Health Organization (WHO) (1). Infertility is known as a major public health concern affecting 8% to 12% of couples of reproductive age all around the world (2). Male-factor infertility is known as the sole contributor to 20% to 30% of infertilities, while it is responsible for 50% of couple infertility cases (3, 4). A wide range of causes is found for male infertility, including varicocele, congenital and acquired urogenital abnormalities, urogenital infections, endocrine disorders, immunologic factors, and sexual dysfunction (5). Despite recent advances, 50% of male infertility cases are idiopathic, without any discernable cause of abnormal sperm parameters (6). Thus, exploring potential risk factors for the early diagnosis and management of idiopathic male infertility is a necessitate.

Some evidence has demonstrated that deficiency of folate (or vitamin B9) possibly plays a role in the etiology of idiopathic male infertility. Folate is a water-soluble vitamin that must be obtained from dietary sources such as leafy green vegetables, fruits, and liver or supplementation with folic acid (7, 8). Various biochemical forms of folate, known as folates, are involved in many one-carbon transfer metabolic pathways like purine and pyrimidine biosynthesis, amino acid metabolism, and methylation reactions (9). Folate deficiency arises from inadequate dietary intake or congenital deficiency of enzymes involved in folate metabolism may affect these metabolic pathways and lead to impaired spermatogenesis (10).

Previous findings from animal studies confirmed the influence of folate deficiency on sperm parameters. Swayne et al

Key point

Folate deficiency probably plays a role in the pathophysiology of idiopathic male infertility.

investigated the influence of folic acid deficiency and supplementation on Sperm parameters. They concluded that folic acid deficiency in adult male mice resulted in reduced sperm number, increased chromatin structure damage, and DNA mutation (11). The results of the study by Ly et al indicated that lifetime feeding of Balb/c mice with folic acid deficient diets caused decreased sperm numbers and alteration of sperm imprinted gene methylation (12). Besides, past interventional studies in human subjects have shown that folate supplementation influences sperm parameters and the reproductive health of males (13, 14). The results of a study by Bentivoglio et al indicated that daily supplementation of 15 mg folinic acid for three months improves spermatozoa number and motility (13). Furthermore, Wong et al in a double-blind, randomized, placebo-controlled study found that co-supplementation of folic acid (5 mg) and zinc sulfate (66 mg) for 26 weeks resulted in an increase of 74% in sperm count among subfertile men (14).

Few studies with contradictory results have investigated the association between serum folate level with sperm parameters and the risk of male infertility in human subjects (14-17). To the best of our knowledge, no previous study has evaluated the correlation between sperm parameters and serum level of folate in Iranian infertile men.

Objectives

The aim of the current study was to investigate the association between folate serum levels and sperm parameters among Iranian males diagnosed with idiopathic infertility.

Patients and Methods

Study design and participants

In this cross-sectional study, we recruited infertile males presenting at the infertility treatment unit (Khanevadeh specialty hospital and Sharifi laboratory, Isfahan, Iran), between March 2020 to July 2021 by convenience sampling method. All males aged 20 to 55 years without diagnosed infertile wives who failed to conceive after 12 months of regular unprotected sexual intercourse were included in the study. Subjects with known causes of infertility (varicocele, infection, and acquired or congenital urogenital abnormalities), endocrine diseases, malignancies, liver and kidney dysfunction, and folic acid or multivitamin supplementation were excluded from the study. Basic characteristics of patients comprising age, current smoking, opium use, weight (kg), height (cm), infertility duration, history of chronic disease such as diabetes mellitus, cancer, and neurologic disorders, a previous history of abdominal surgery, and drug therapy for infertility were collected using a standard checklist.

Semen analysis

After three days of sexual abstinence, semen samples were collected by masturbation in a sterile container. Sperm concentration, volume, vitality, progression/total motility, morphology, and agglutination as well as semen leukocyte count were assessed based on the WHO guidelines (18).

Sperm chromatin dispersion test

The sperm chromatin dispersion test, as described by Frenández et al and Derakhshan et al, was applied to examine sperm DNA fragmentation level (19,20). At least 500 sperm cells per slide were assessed under bright field microscopy and classified base on halo size into the following categories: spermatozoa with big-sized halo, medium-sized halo, small-sized halo, spermatozoa without halo, and degraded spermatozoa without a halo. Spermatozoa with medium- and big-sized halos were considered non-fragmented; while those with small-size halo, no halo, and degradation were taken into account as fragmented. DNA fragmentation index was calculated as the ratio of fragmented sperms to the total number of sperms.

Aniline-blue test (sperm chromatin maturity assay)

The assessment of sperm chromatin maturity was conducted by the aniline-blue test (AB). Briefly, air-dried smears of semen samples were fixed at room temperature in 3% glutaraldehyde solution in 0.2 M phosphate buffer (pH: 7.2) for 30 minutes. Afterward, each smear was stained with a mixture of 5% aqueous aniline blue and 4% acetic acid (pH:3.5) for 5 minutes. One hundred spermatozoa per slide were counted under light microscopy. Unstained or pale-blue stained (AB-) spermatozoa were considered normal and dark blue stained spermatozoa (AB+) were considered abnormal. The percentage of abnormal sperm cells was reported.

Serum folate level

Fasting blood samples were obtained from all participants for the assessment of serum folate levels. Then blood samples were centrifuged at 3000 rpm for 10 minutes, where the serum was separated and stored at -80°C for further analysis. Folate concentration was measured by electrochemiluminescence in a 2010 Elecsys automated equipment (Roche Diagnostics). The serum folate levels were classified as deficient (<3 ng/mL) and normal (≥ 3-17 ng/mL).

Statistical analysis

Continuous and categorical data were presented as mean ± standard deviation and frequency respectively. Normality of continuous variables was evaluated through Kolmogorov-Smirnov test and Q-Q plot. Non-normally positive skewed data were subjected to logarithmic transformation. Basic clinical and demographic continuous and categorical variables were compared between infertile men with and without folate deficiency by independent samples *t* test and chi-square test, respectively. Continuous sperm parameters were compared between infertile men with and without folate deficiency using independent samples *t* test and analysis covariance (ANCOVA) by adjusting possible confounders. We also compared the distribution of categorical sperm parameters between infertile men with and without folate deficiency through chi-square test and their association was quantified by estimating odds ratio (OR) along with 95% confidence interval for OR through logistic regression model both in univariate and multivariable models (adjustment was made for potential confounders).

Results

In total, 100 patients were screened for possible inclusion in the study, of whom 78 met the inclusion criteria and 8 were excluded because of unwillingness to cooperate in the study. Thus, the final analysis was performed on 70 infertile males comprising 46 subjects with sufficient serum folate level and 24 subjects with deficient serum folate level. Patients had a mean age of 34.49±6 years and a mean body mass index (BMI) of 27.02±3.1 kg/m^2. Furthermore, the mean duration of infertility was 1.43±0.9 years.

The basic characteristics of infertile males according to serum folate levels are shown in Table 1. The mean age (33.33 ± 5.43 versus 36.71 ± 6.54, P = 0.024) and infertility duration (1.22 ± 0.85 versus 1.83 ± 0.89, P = 0.008) was significantly lower in subjects with sufficient serum folate level compared to subjects with deficient serum folate level. Subjects with sufficient serum folate level were less likely to have a previous history of abdominal surgery (P = 0.04). No significant difference was observed regarding other basic characteristics across categories of serum folate levels.

As shown in Figure 1, sperm parameters were compared between categories of serum folate level. Subjects with sufficient serum folate level had a significantly higher mean of sperm concentration (59.57 ± 31.79 versus 31.41 ± 20.86; P < 0.001), vitality (85.78 ± 11.27 versus 76.50 ± 21.25; P = 0.02), progressive motility (31.17 ± 5.14 versus 20.08 ± 10.76; P < 0.001), total motility (51.89 ± 8.62 versus 42.50 ± 14.69; P = 0.001), sperm DNA fragmentation assay (SDFA) (15.11 ± 4.72 versus 22.96 ± 4.53; P < 0.001), and sperm chromatin maturity assay (SCMA) (23.54 ± 6.81 versus 28.96 ± 5.74; P = 0.001). Furthermore, the difference in sperm parameters across categories of serum folate level remained significant after adjustment for age and infertility duration and history of chronic disease and abdominal surgery. However, no significant difference was observed between two groups regarding sperm volume (P > 0.05).

Comparing the frequency of abnormal sperm agglutination, morphology, and semen leukocyte count between the two groups indicated that the frequency of abnormal morphology was significantly higher in subjects with deficient serum folate level (P = 0.002). However, no significant difference was observed across categories of serum folate level in terms of abnormal sperm agglutination (P = 0.06), and semen leukocyte count (P > 0.99; Table 2). To quantify the association between serum folate level and the mentioned semen parameters and univariate and multivariable logistic regression test were performed. No significant association was observed between serum folate level and abnormal sperm agglutination in the non-adjusted model (OR: 0.23, 95% CI: 0.05-1.15). Although sufficient serum folate level was associated with 91% decreased risk of abnormal sperm agglutination in the adjusted model (OR: 0.09, 95% CI: 0.01-0.65). In addition, subjects with sufficient serum folate level had 89% and 88% reduced risk of abnormal morphology both in the crude (OR: 0.11, 95% CI: 0.02-0.51) and adjusted (OR: 0.12, 95% CI: 0.02-0.60) models. No significant association was found between abnormal semen leukocyte count and folate serum levels in the crude (OR: 1.01, 95% CI: 0.37-2.78) and adjusted model (OR: 0.79, 95% CI: 0.27-2.33; Table 2).

Discussion

The present study indicated that there was a significant association between serum folate level and some sperm parameters such as sperm concentration, morphology, vitality, progressive motility, total motility, SDFA, and SCMA. However, there was no significant relationship

Table 1. General characteristics and sperm parameters of subjects with sufficient serum level of folate compared to subjects with deficient serum level of folate

Variable	Folate serum levels		P value[a]
	Sufficient (n=46)	Deficient (n=24)	
Age (y)	33.33±5.43	36.71±6.54	0.024
Body mass index (kg/m^2)	27.12±3.08	26.84±3.09	0.718
Infertility duration (y)	1.22±0.85	1.83±0.89	0.008
Current smoking	13%	8.3%	0.71
Opium use	13%	8.3%	0.71
History of chronic disease	13%	22.9%	0.12
Drug therapy for infertility	8.7%	20.8%	0.26
History of abdominal surgery	2.2%	16.7%	0.04

Values are Mean ± SD; unless indicated.

[a] Obtained from independent samples t test and χ2 test for quantitative and categorical variables, respectively.

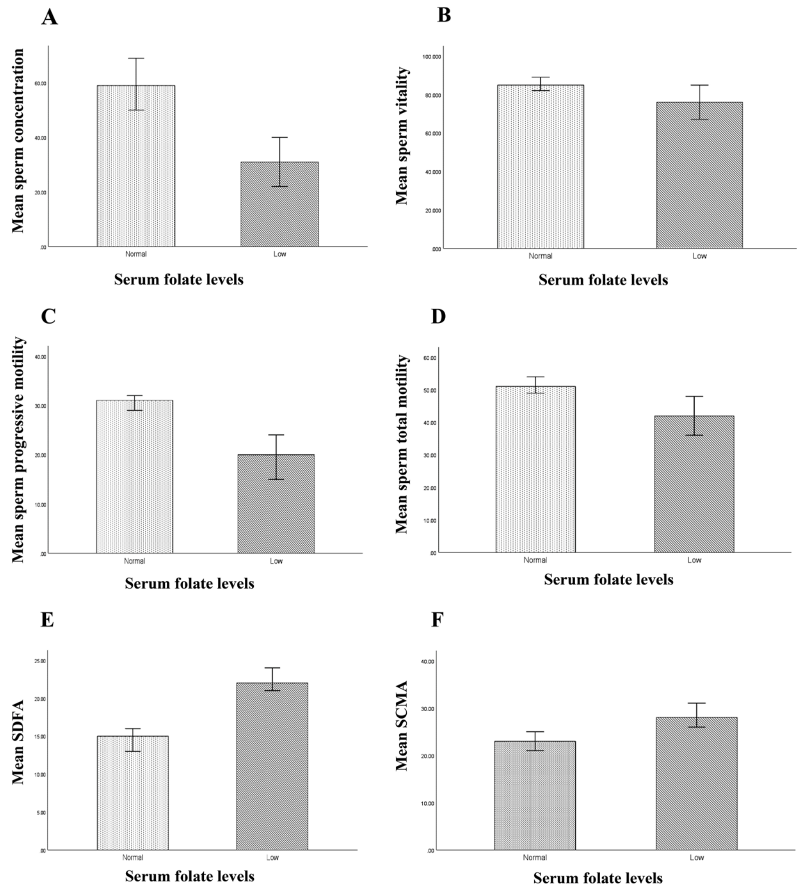

Figure 1. The comparison of sperm parameters between subjects with normal serum folate level and low serum folate levels. (A) Concentration (B) vitality, (C) progressive motility, (D) total motility, (E) SDFA, and (F) SCMA.

between semen leukocyte count and folate serum levels even after adjustment for confounding variables. To the best of our knowledge, this was the first study that investigated the association between serum folate level and sperm parameters in Iranian males with idiopathic infertility.

There is some evidence that lifestyle factors such as smoking, alcohol drinking, obesity, psychological conditions, and diet play an important role in the pathogenesis of male infertility (21,22). Our findings have suggested that the recommendations for increased dietary intake of folate-rich foods such as dark green leafy vegetables, orange juice, and liver as well as acid folic supplementation are possibly beneficial approaches to treat idiopathic male infertility through improving sperm parameters.

Few numbers of previous observational studies with contrary results have investigated the effect of folate on sperm parameters in human subjects. Tariq et al in a cross-sectional study of 274 Pakistani males showed that a higher serum level of folate in fertile males compared to infertile males. Similar to our findings, the researchers found a significant positive association between serum folate level and some of sperm parameters such as total sperm count, motility, and morphology (23). Conversely, in another study by Murphy et al serum folic acid levels were significantly lower in infertile men compared to fertile men, but no correlation was found between serum

Table 2. Association between serum folate level and abnormality in sperm parameters

	Serum folate level			Sufficient serum folate versus Deficient serum folate	
	Sufficient	Deficient	P value[a]	Non-adjusted Odds ratio (95% CI for OR)	Adjusted odds ratio (95% CI for OR)[b]
Abnormal sperm agglutination	28.9%	8.7	0.07	0.23 (0.05-1.15)	0.09 (0.01-0.65)
Abnormal sperm morphology	54.3	91.7	0.001	0.11 (0.02-0.51)	0.12 (0.02-0.60)
Abnormal semen leukocyte count	41.3	41.7	>0.99	1.01 (0.37-2.78)	0.79 (0.27-2.33)

[a] Obtained from χ^2 test.
[b] Adjusted for age, infertility duration and history of chronic disease and abdominal surgery.

folate level and sperm parameters in the study (24). The conflicting results observed from these studies could be due to differences in the study population or the method of conducting the study. Some interventional studies have also investigated the effect of folic acid supplementation on sperm parameters. However, there is no consensus regarding the therapeutic effect of folic acid on sperm parameters. Although the results of some studies indicate that supplementation with folic acid improves some sperm parameters such as sperm count and motility among infertile males (13,14), other studies failed to show a significant effect of folic acid on sperm parameters and semen quality in infertile males (11,16,25). Thus, further studies on a large sample of patients are required to detect the relationship between serum folate levels and sperm parameters.

The relation between serum folate level and sperm parameters can be explained by several mechanisms. Folate is involved in the synthesis of nucleic acids and proteins through the synthesis of S-adenosylmethionine through the one-carbon metabolic pathway. Thus folate deficiency can influence spermatogenesis through altering gene expression (11,26). Furthermore, previous reports suggest that oxidative stress, the imbalance between reactive oxygen species (ROS) and antioxidant compounds, plays a significant role in male infertility due to the development of abnormal sperm parameters as result of lipid peroxidation and DNA damage (10,27). Therefore, folate probably has a positive effect on sperm parameters through its antioxidant properties. While its deficiency seems to defect spermatogenesis by increasing the level of homocysteine and associated inflammatory cytokines (28). However, future interventional studies are required to confirm the assumption.

Conclusion

In summary, we found a significant association between serum folate level and infertility among Iranian infertile males. However, prospective large-scale population-based studies are needed to confirm the finding.

Limitations of the study

The present study has several limitations that should be addressed. First, the cross-sectional design of the study cannot represent the causal link between serum folate and sperm parameters in infertile males. Second, the present study was performed on a relatively small population of infertile males. Third, although a number of known confounding variables such as age, BMI, chronic diseases, smoking and tobacco use have been controlled in this study, uncontrolled confounders such as physical activity, socioeconomic status, psychological, occupational and environmental factors may affect our results. However, the results of the study can be used for designing future prospective population-based studies to explore the association between folate level and male infertility.

Acknowledgements

We would like to thank all patients and clinical staff of Khanevadeh hospital for their excellent collaboration in the project.

Authors’ contribution

Conceptualization: Marz D.
Methodology: Marz D.
Validation: Mary D
Formal analysis: SSA
Investigation: PM
Resources: Mary D
Data curation: SSA
Writing–original draft preparation: SSA
Writing–review and editing: Marz D
Visualization: Mary D
Supervision: Marz D, Mary D, PM
Project administration: Marz D
Funding acquisition: Mary D

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upo reasonable request.

Conflicts of interest

There is non conflict of interest to be declared.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The study protocol was approved by the ethics committee of Isfahan University of Medical Sciences, Isfahan, Iran (Code of ethics: IR.MUI.REC.1399.570). All subjects were informed about the study objectives and provided a written informed consent before taking part in the study. Ethical issues (including plagiarism, data fabrication and double publication) have been completely observed by the authors.

Funding/Support

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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