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Original article

Utility of Fibroblast Growth Factor 21 as a Novel Marker in Cases of Idiopathic Male Infertility

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Abstract:

Background: The male factor accounts for approximately 50% of infertility cases. Fibroblast growth factor 21 (FGF21) has been implicated in the female reproduction, raising the possibility of its role in male fertility. Aim: To assess serum and seminal FGF21 levels in infertile men. **Materials and methods:** Participants were separated into two groups: infertile men (n=60) and normally fertile men (n=60). They were subjected to a history and genital examination. The laboratory tests included serum total testosterone, follicle-stimulating hormone, luteinizing hormone, and standard sperm analysis. FGF-21 levels in serum and sperm were determined using the ELISA method. **Results:** The mean duration of infertility in patients was 4.3 ± 2.7years. Patients had significantly reduced levels of FGF21 in sperm and serum compared to controls (P value < 0.001). FGF21 levels were considerably lower in semen than serum in both groups (P value < 0.001). Serum and seminal FGF21 levels showed a substantial correlation with sperm concentration, motility, and morphology (P value < 0.001). **Conclusion:** FGF-21 could have an important role in the pathophysiology of idiopathic male infertility.

Keywords: Fibroblast growth factor 21; Male infertility; Semen

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Introduction

Infertility is a common problem affecting 13-15% of partners worldwide. Male factor represents half of the cases. Several causes of male factor infertility have been identified, including genetic and hormonal disorders. However, nearly one third of infertile men exhibit abnormal sperm parameters with no definite cause, even in the most comprehensive investigations, which is defined as idiopathic infertility [1, 2].

The fibroblast growth factors (FGFs) comprise 22 members distributed into seven subfamilies [3]. Data indicate that FGF21 is involved in female fertility problems, implying that it is also involved in male fertility [4]. Receptors for FGF have been detected in the seminiferous tubules of mice [5], as well as in human testis [6]. These FGFs have been shown to have paracrine and local activities during testis development, such as stimulating proliferation and survival or contributing to the establishment of the testicular interstitial compartment [7].

There are few published studies on the FGF21 subfamily. Animal investigations demonstrated that deletion of FGF21 gene resulted in increased quantity of apoptosis of testicular germ cells. The data also support the FGF21 hormone's regulatory role in the male reproduction. But, the effects of FGF21 in the sperm have not been reported [8]. Idiopathic male factor infertility is a big concern for physicians. Therfore, this study aimed to explore the relationship between FGF21 levels in semen and serum with semen parameters.

Materials and Methods

Study design and population

This cross-sectional study was carried out in the Andrology Outpatient Clinic, Faculty of Medicine, Benha University Hospital, from May 2023 to December 2024. This was carried out with the agreement of Benha University's Faculty of Medicine Ethics Committee (MS 37-3-2023). All subjects provided informed written consents. The study comprised infertile males with aberrant semen parameters. However, those with a clear reason of infertility, such as hormonal imbalances or a varicocele, were eliminated. Furthermore, individuals with liver problems, diabetes mellitus, or autoimmune illnesses, or those who had undergone therapy for infertility in the previous three months- were not suitable to share in this study.

Methods:

The study comprised 60 men with primary infertility and 60 fertile men as controls. Participants underwent a general checkup, including obtaining their history, measuring their body mass index (kg/m²), and assessing secondary sexual traits. Simultaneously, a genital examination was performed to rule out any potential causes of infertility, such as a varicocele. Scrotal ultrasonography was used for the imaging. Hormonal tests were performed to assess total testosterone, follicle-stimulating hormone, and luteinizing hormone. Semen was collected and analysed in accordance with World Health Organisation (WHO) guidelines using computer-assisted sperm analysis [9]. FGF21 was also evaluated in semen and serum using the Human FGF21 ELISA kit SunRed, China (Catalogue No. 201-12-1984, sensitivity: 5.524 ng/L, assay range: 6-1800 ng/L).

Statistical analysis:

The data were analyzed with the Statistical Package for Social Science version 26. The quantitative data was given as mean and standard deviation (±SD), whereas qualitative data was reported as number and percentage. The Student's t-test, Mann-Whitney U-test, and Chi-square test were used to make statistical comparisons between groups. The Pearson correlation coefficient test was performed to correlate various factors. Some of the analyzed parameters were entered into a regression analysis model to identify which factors are regarded important predictors. The optimal discriminating point was determined by examining the sensitivity

and specificity at several cutoff points using receiver operating characteristic (ROC) curve analysis. P-value < 0.05 was considered significant.

Results

Age, BMI, and smoking did not show significant difference between patients and controls (P value > 0.05 for all). The mean duration of infertility in patients was 4.3 ± 2.7 years. In patients, sperm concentration and motility were considerably lower than in controls (P < 0.001). The patient group had a normal sperm morphology index, although considerably less than the controls (P value < 0.001). Patients had significantly reduced levels of FGF21 in semen and serum compared to controls (P value < 0.001). FGF21 levels were considerably lower in semen compared to serum in both groups (P value < 0.001), Table (1).

FGF21 showed a substantial positive connection with sperm concentration, motility, and morphology index (P value < 0.001 for all). However, there was no significant relationship between FGF21 and age, BMI, or duration of infertility (P value > 0.05 for all),Table 92).

The multivariate regression analysis revealed that FGF21 levels in semen and serum were significant predictors of infertility, Table (3).

The receiver operating characteristic (ROC) curve research found that measuring FGF21 levels in semen is more accurate than in serum for predicting infertility in men (Area Under the Curve = 0.8 and 0.7, respectively), Table (4), Figure (1).

Table 1. Comparison of the characteristics of the study groups

Characteristics		**Patient (n=60)**	**Control (n=60)**	**Test of significance**	**P-value**
Age (years)		29.80 ± 6.19	28.13 ± 2.97	t =1.9	0.06
BMI (kg/m2)		26.13 ± 4.06	25.27 ± 2.64	t =1.4	0.2
Smoking (n, %)	**Non-Smoker**	14 (23.3%)	22 (36.6%)	X^2 =2.5	0.111
	Smoker	46 (76.7%)	38 (63.3%)		
Duration of Infertility(Years) Mean+SD Range		4.3 ± 2.7 2-12	-------	-----	------
FSH (ng/ml)		5.6 ± 1.9	5.4 ± 1.8	t =0.6	0.55
LH (ng/ml)		4.9±1.7	4.7±1.5	t= 0.68	0.749
Total testosterone (ng/ml)		570±125	580±120	t=0.44	0.65
Sperm Concentration (million/ml)		15.5 ± 3.7	70.87 ± 47.29	U=8.9	<0.001*
Sperm Motility (mean %±SD)	**Progressive**	18.15 ± 8.19	39.36 ± 5.59	t =16.6	<0.001*
	Non-Progressive	17.28 ± 8.22	29.04 ± 6.74	t =8.6	<0.001*
Sperm Morphology Index (%)		39.81 ± 8.07	59.56 ± 8.83	t =12.8	<0.001*
FGF21 (ng/ml)	**Semen**	42.30 ± 5.82	87.55 ± 7.63	t =36.5	<0.001*
	Serum	81.60 ± 6.33	182.05 ± 6.06	t =88.7	<0.001*
	P-value	<0.001*	<0.001*	**----**	**----**

BMI: Body Mass Index, SD: Standard deviation, FGF21: Fibroblast growth factor 21, X^2:Chi square test, t: Student t test, U:Mann-Whitney U test, * significant P-value ≤ 0.05.

Table 2. Correlations between FGF21 and different variables

	Semen FGF21		Serum FGF21	
	R	**P-value**	**R**	**P-value**
Age	-0.16	0.07	-0.15	0.09
BMI	-0.13	0.17	-0.12	0.21
Duration of Infertility	0.1	0.96	0.12	0.36
Sperm Concentration	0.33	<0.001*	0.36	<0.001*
Progressive sperm motility	0.74	<0.001*	0.76	<0.001*
Non-Progressive sperm motility	0.43	<0.001*	0.47	<0.001*
Normal Sperm Morphology	0.59	<0.001*	0.60	<0.001*
serum FGF21	0.95	<0.001*		

BMI: Body Mass Index, FGF21: Fibroblast growth factor 21, R: correlation coefficient, * Significant P-value ≤ 0.05.

Table 3. Regression analysis for prediction of male infertility

	Univariate analysis			Multivariate analysis		
	OR	**95% CI**	**P-value**	**OR**	**95% CI**	**P-value**
Age	1.08	0.99-1.18	0.07	1.07	0.92-1.23	0.4
BMI	1.07	0.97-1.2	0.1	1.16	0.99-1.35	0.1
Semen FGF21	1.02	1.01-1.03	<0.001*	1.02	1.01-1.03	<0.001*
Serum FGF21	1.03	1.01-1.04	<0.001*	1.03	1.01-1.04	0.001*

BMI; Body Mass Index, FGF21: Fibroblast growth factor 21, OR:Odds Ratio, CI:Confidence Interval, * Significant P-value ≤ 0.05.

Table 4. Diagnostic accuracy of FGF21 for prediction of infertility in men

	AUC	**95% CI**	**Cutoff (ng/ml)**	**Sensitivity**	**Specificity**
Semen FGF21	0.8	0.7-0.9	103.7	80	86.7
Serum FGF21	0.7	0.5-0.8	104.03	63.3	86.7

FGF21: Fibroblast growth factor 21, AUC: Area Under the Curve, CI: Confidence Interval

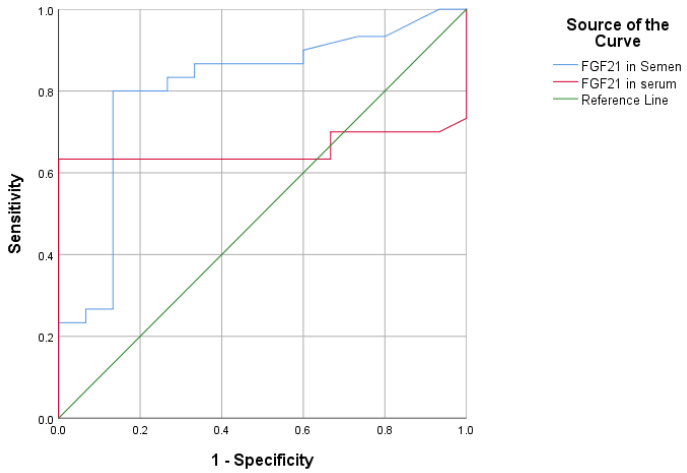

Figure 1. Roc curve for semen and serum FGF21 in prediction of infertility

Discussion

The current study found that infertile males had lower levels of both serum and seminal FGF21 than normal subjects. Seminal levels were significantly less than serum levels in patients and controls. Similarly, Bourdon et al. [4] found that FGF21 content in the seminal fluid was about 20 times lower than in the plasma.

Two possibilities were proposed on the genesis of seminal FGF21. First, blood FGF21 is produced primarily in the liver and passes the blood-testis barrier, either passively or actively; second, it could be secreted by several organs of male reproductive tract. The epididymis, seminal vesicles, and prostate gland produce the majority of semen proteins. Immunochemistry against FGF21 in the male reproductive tract revealed expression in Leydig cells, epididymal epithelium, and seminal vesicles. But, the spermatozoa did ot show positive staining [(4)].

FGF21 in hepatocytes is known to be controlled by both hunger and peroxisome proliferator-activate receptors (PPARa) activation. PPARa has previously been detected in prostatic epithelium [(10, 11)]. Obese men have significantly higher plasma baseline FGF21 levels, which may be associated with reduction in testosterone synthesis [(12)].

The present study found a strong positive link between FGF21 (semen or serum) and sperm concentration, motility, and normal sperm morphology index. In contrast, another study found no significant association between FGF21 levels and the sperm concentration, and motility [(4)].

The current findings can be explained by the fact that FGF21 has an essential role in metabolic control. It plays an important role in glucose homeostasis, insulin sensitivity, ketogenesis, and normal spermatogenesis. FGF21 maintains balance between germ cell death and spermatogenesis by inhibiting the activation p53 via murine double minute 2 (MDM2) and protein kinase B (AKT). FGF21 deficiency promotes germ cell death in diabetes patients [(13)].

The sperm motility was investigated after the exposure of human sperms to FGF21 for 30 minutes at 37°C. At FGF21 doses of 0.1 ng/mL and higher, progressive motility increased dramatically when compared to the control condition. Preincubation with the FGFR inhibitor proved effective in eliminating the activation effect of FGF21. Furthermore, FGF21 alleviated oxidative stress in human sperm in a dose-dependent manner [(4)].

In the same context, Chu et al. [(14)] confirm the current findings by demonstrating that in vitro treatment with FGF21 dramatically boosted sperm motility and ATP levels, indicating that FGF21 positively influences the activity and quality of human spermatozoa characteristics.

The univariate logistic regression analysis revealed that semen FGF21 and serum FGF21 were the only significant predictors of infertility, but age and BMI were not. The multivariate logistic regression analysis revealed that semen FGF21 and serum FGF21 were significant predictors of infertility. Furthermore, the receiver operating characteristic (ROC) curve demonstrated that seminal FGF21 performed better than serum FGF21 in predicting infertility (AUC=0.8 and 0.7, respectively).

Limitations include the small sample size, single-center methodology, and the need for more immunohistochemistry investigations.

Conclusions:

FGF21 is substantially linked with sperm parameters and might have a role in the pathogenesis of idiopathic male infertility.

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Conflict of Interest: Nil

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