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Research Article

Seminal TEX101 May Predict Seminal Sperm Recovery after Varicocelectomy in Nonobstructive Azoospermic Patients with Varicocele

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Background. Infertile men with nonobstructive azoospermia (NOA) associated with varicocele may benefit from varicocele repair with recovered sperms in semen. Currently, there is no clinically applied method to predict the success of seminal sperm recovery in this subset of azoospermic men. *Objective*. We aimed to evaluate the predictive role of the seminal testis expressed protein (TEX101) in sperm recovery in the semen of NOA men with palpable bilateral varicocele after microsurgical varicocelectomy. *Patients and Methods*. This prospective cross-sectional cohort study included 40 patients with NOA and bilateral varicocele. Seminal TEX101 levels were measured followed by subinguinal microsurgical varicocele repair. Two seminal analyses were carried out at 3- and 6-month follow-up periods to evaluate seminal sperm recovery. *Results*. After varicocele repair, sperms were recovered in the semen of 10/40 patients (25%) along the follow-up periods (seven patients after 3 months and additional three patients after 6 months). The preoperative median seminal TEX101 level was significantly higher in NOA men with seminal sperm recovery compared with NOA patients without seminal sperm recovery (13.5 vs. 9.8 ng/ml, $p = 0.014$). Besides, the serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) median levels were significantly higher in NOA men without seminal sperm recovery compared with NOA men with seminal sperm recovery ($p = 0.001$, $p = 0.01$). There were nonsignificant differences comparing the preoperative testicular volume or serum testosterone levels between the two investigated groups ($p = 0.072$, $p = 0.272$). A cutoff value of 9.9 ng/ml was demonstrated to have preoperative TEX101 prediction of seminal sperm recovery (sensitivity of 90% and specificity of 57%). *Conclusion*. Preoperative seminal TEX101 could be considered as a biomarker for sperm recovery for seminal sperm recovery in NOA cases associated with varicocele after its surgical repair. Further work at this point with larger number of cases should be carried out to verify its potential importance.

1. Introduction

Azoospermia, the absence of spermatozoa in the semen, is recognized in 1% of all men and in 10%–15% of whole infertile men. It is classified into obstructive and nonobstructive azoospermia (NOA) [1, 2]. Histopathologically, NOA is classified into hypospermatogenesis (HS), maturation arrest (MA), and Sertoli cell-only syndrome (SCO) [3]. Nowadays, testicular sperm extraction (TESE) is the principal process for NOA management hoping to retrieve viable spermatozoa for subsequent intracytoplasmic sperm injection (ICSI) procedure. Restoring sperm recovery in the semen was reported in some cases after medical treatment and/or varicocelectomy [4, 5].

Varicocele can affect spermatogenesis by different mechanisms [6–8]. Varicocele repair in NOA cases aims to alleviate its harmful impact on the testes to recover its spermatogenic activity with subsequent presence of spermatozoa in the semen or to increase the chances for successful TESE for ICSI process [9, 10].

As early as 1955, Tulloch [11] reported the first case of restoration of spermatogenesis after varicocelectomy. Later, subsequent meta-analyses reported that around 40% of

selected patients with NOA had improvement in testicular functions and spermatozoa were found in ejaculate after varicocelectomy [4, 5, 12]. It was speculated that varicocelectomy may enhance the testicular microenvironment with improved spermatogenic activity in some patients. Besides, it has been suggested that the consequences of varicocele repair in NOA cases with either HS or late MA have better prognosis in comparison to NOA cases with either early MA or SCO cases [13, 14].

Currently, there are no particular noninvasive diagnostic markers for defining that prediction. In this context, it is reasonable to study the semen, which comprises fluid from the testis and the genital tract and can be accessed with comparative ease as an emerging source for such potential biomarkers.

Seminal plasma human testis-expressed sequence 101 (TEX101) is a protein that is synthesized exclusively by testicular germ cells [15–18]. It is a membrane glycosylphosphatidylinositol-anchored protein with a UPAR/Ly6 domain, indicating a similar structure as urokinase-type plasminogen activator receptor. This membrane protein is detected in developing spermatogenic cells in the testis but not in the interstitial cells of the testis and Sertoli cells. In the caput epididymis, TEX101 is shed from spermatozoa [18–21]. Saleem et al. [20] pointed out to the significantly lower seminal TEX101 levels in azoospermic men compared to idiopathic oligoasthenoteratozoospermic patients. Moreover, seminal levels of TEX101 have been demonstrated to categorize different azoospermia forms based on testicular histopathology and to predict TESE outcome [15, 22].

This study aimed to assess the role of seminal TEX101 for restoration of seminal sperm recovery after microsurgical varicocelectomy in a cohort of NOA patients.

2. Patients and Methods

This prospective cross-sectional cohort study was conducted at the University hospital after informed consent, as well as institutional review board (IRB) approval (No. NCT04397887). Overall, 40 patients with NOA azoospermia and clinically palpable bilateral varicocele were included. Azoospermia was confirmed by two semen analyses performed according to the World Health Organization (WHO) guidelines. Exclusion criteria were testis volume <6 ml, subclinical varicocele, evident obstructive azoospermia, history of undescended testis, chemotherapy/radiotherapy, testicular surgery, or chromosomal abnormalities.

These men were subjected to full history taking, genital clinical examination, semen analysis, serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, estradiol, and prolactin evaluation. Scrotal ultrasound was carried out to confirm clinically palpable varicocele, with at least one vein seen having a diameter of 2 mm on each side using ultrasound device (Siemens Sonoline G50, NY, USA) by a linear array transducer possessing a minimum frequency of 10 MHz.

2.1. Surgical Procedure. Microsurgical subinguinal varicocele repair was done by the same team.

2.2. Measurement of the Human TEX101 in Semen. The human TEX101 was assayed by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Wuhan Fine Biotech Co., Ltd., China). This kit was based on sandwich ELISA technique. Anti-TEX101 antibody was precoated onto 96-well plate. Biotin-conjugated antibody was used as detection antibodies. Test samples and biotin-conjugated detection antibody were added to the wells subsequently and washed with wash buffer. Horseradish peroxidase (HRP)-streptavidin was added, and unbound conjugates were washed away with wash buffer. Tetramethylbenzidine (TMB) substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The intensity of yellow is proportional to the target amount of sample captured in plate.

2.3. Follow-Up. Postoperative semen analyses at 3 and 6 months were carried out to assess cases with sperm recovery.

2.4. Statistical Analysis. It was carried out with the SPSS program (version 23) software (SPSS Inc., Chicago, IL, USA). The data were presented as medians and ranges where Mann–Whitney U test was used in comparisons. Receiver operating characteristic (ROC) curve test was used to calculate a cutoff value and the diagnostic indices (sensitivity and specificity). $p<0.05$ was set as being statistically significant.

3. Results

This study comprised 40 patients with azoospermia and bilateral palpable varicocele. Grade III varicocele was found in left side in 12 patients with grade II in the right side, while the remaining patients (28/40) had bilateral grade II varicocele. After varicocelectomy, spermatozoa were recovered in the ejaculate of 10/40 (25%) patients through the follow-up (seven patients at the 3-month follow-up and additional three patients at 6-month follow-up). Sperm concentration in these patients was <1,000/ml in six patients, $0.001–5\times10^6$/ml in three patients, and one patient had a sperm concentration of 14.2×10^6/ml. The preoperative median seminal TEX101 level was significantly higher in NOA men with seminal sperm recovery compared to NOA men with no seminal sperm recovery ($p=0.014$). Besides, the serum FSH and LH median levels were significantly higher in NOA men with no seminal sperm recovery compared to NOA men with seminal sperm recovery ($p=0.001$, $p=0.01$). There were nonsignificant differences comparing the preoperative testicular volume or serum testosterone levels between the two groups ($p=0.072$, $p=0.272$) (Table 1). Using ROC curve, the area under curve (AUC) was 0.76. A cutoff value of 9.9 ng/ml was demonstrated to have preoperative TEX101 prediction of seminal sperm recovery (sensitivity of 90% and specificity of 57%) (Figure 1).

4. Discussion

In the current study, seminal sperm recovery was demonstrated postvaricocelectomy in 10/40 (25%) of the NOA patients along 6-month follow-up. The preoperative median seminal TEX101 level was significantly higher in NOA men

Table 1: Preoperative data of the investigated NOA cases (median, range).

	Seminal sperm recovery	No seminal sperm recovery	*P*-value
n	10	30	
Age (years)	30.5 (23–40)	29 (18–43)	0.569
Right testis volume (ml)	12.8 (8.9–14.7)	11.6 (6.1–15)	0.109
Left testis volume (ml)	11.3 (7.8–13.1)	9.8 (6–16.1)	0.432
Mean testis volume (ml)	12.3 (8.3–13.9)	10.6 (6.2–14.5)	0.272
Right vein diameter (mm)	2.8 (2.1–3.8)	2.7 (2.2–3)	0.109
Left vein diameter (mm)	3.4 (2.9–5.9)	3.1 (2.4–4.7)	0.198
Serum FSH (mIU/ml)	5.1 (3.5–9.2)	13.9 (3.5–31.2)	0.001*
Serum LH (mIU/ml)	4.2 (2.4–6.5)	6.3 (2.7–27)	0.010*
Serum total testosterone (nmol/L)	5.1 (2.2–13)	4.5 (1.9–10.8)	0.818
Serum prolactin (ng/ml)	9.9 (4.7–19.2)	14.8 (2.4–23)	0.414
Serum estradiol (pm/ml)	24.4 (14–42)	27.4 (12–50)	0.569
Seminal TEX101 (ng/ml)	13.5 (7.5–22.3)	9.8 (2.8–18.2)	0.014*

*Significant statistical difference.

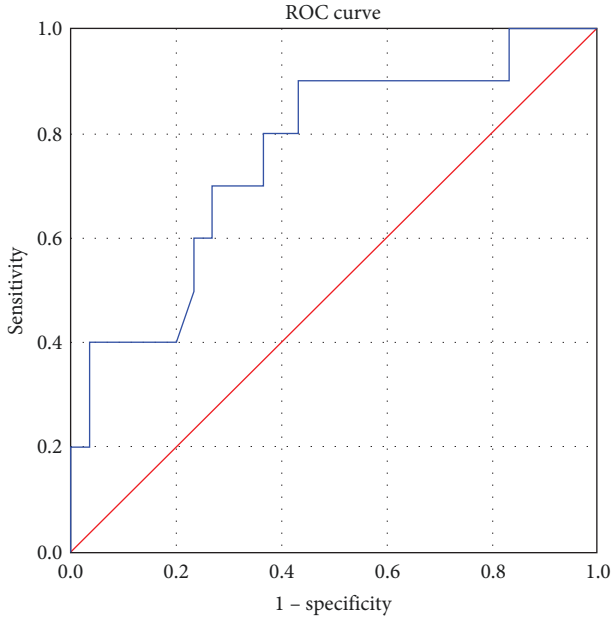

Figure 1: Receiver operating characteristic curve of preoperative seminal TEX101 levels.

with seminal sperm recovery compared to NOA men with no seminal sperm recovery. However, the majority of these patients (9/10) had a sperm concentration <5 million/ml and semen cryopreservation was recommended due to possibility of relapsing to azoospermia [23].

Regarding seminal sperm recovery after varicocele surgical repair in NOA cases, Weedin et al. [5] collected 11 previous studies showing that 91/233 (39.1%) of these patients recovered motile spermatozoa in the ejaculate with a mean postoperative follow-up of 13.3 months. In 2011, Schlegel and Goldstein [4] presented more studies showing that NOA patients could recover spermatozoa in at least one semen analysis in 36% of the cases (range 21%–56%). In 2013, Elzanaty [24] presented eight studies that associated varicocele repair with recovered motile spermatozoa in the semen in 55% of the investigated NOA cases. Besides, in 2016, Esteves et al. [12] included 16 studies that reported presence of seminal sperms in 43.9% (151/344) of the NOA cases after varicocelectomy with a mean postoperative follow-up of 12.36 ± 5.49 months. In the current work, we represented 24 studies about the effect of varicocelectomy on NOA cases that demonstrated seminal sperm recovery in 243/831 (29.2%) (range 13.8%–56.3%) (Table 2). Despite these supporting data for performing varicocelectomy for men with NOA and palpable varicocele, the current guidelines by American Society for Reproductive Medicine (ASRM) and American Urological Association (AUA) do not recommend varicocelectomy for this subset of patients [47], while the European Association of Urology (EAU) suggested varicocelectomy for patients with NOA and varicocele, as it may avoid the need for TESE due to appearance of sperms in the ejaculate [48, 49].

Generally, spermatogenesis can occur in a damaged testis, causing focal areas, or patches, of sperm production in an organ lacking germ cells. In this context, NOA patients with SCO and MA have worse prognosis compared to those with either late MA or HS. Therefore, it has been demonstrated that the success rates in patients with MA (42.1%) or HS (54.5%) were significantly higher than in SCO (11.3%) cases [5].

TEX101 is a membrane protein, which is solely expressed in spermatogenic cells and sperms. This protein is cleaved from the cell membrane of the sperm during spermiogenesis in epididymis. Thus, in men with obstructive azoospermia or Sertoli cell only, TEX101 is not detected in seminal plasma [22].

In the current study, preoperative seminal TEX101 AUC was 0.76 with cutoff value of >9.9 ng/ml with 90% sensitivity and 57% specificity in predicting seminal sperm recovery. In their study, Drabovich et al. [22] reported that seminal TEX101 AUC was 1.0 with 95% sensitivity and 100% specificity. They also pointed out that seminal TEX101 can distinguish between SCO cases with a value of <5 ng/ml and MA or HS cases (5–120 ng/ml) that are recommended for TESE with high chances of successful sperm retrieval. Korbakis et al. [15] studied sperm retrieval rates of TESE in NOA cases varied according to the different histological

Table 2: Seminal sperm recovery in NOA cases after varicocelectomy in different studies (*n*, %).

	Author(s) Year	Reference (*n*)	Country	Mean age (years)	NOA cases (*n*)	Seminal sperm recovery (*n*)	%
1	Czaplicki et al., 1979	[9]	Poland	30.3	33	12	12/33 (36.4%)
2	Matthews et al., 1998	[25]	USA	35	22	12	12/22 (54.5%)
3	Kim et al., 1999	[26]	USA	25	28	12	12/28 (42.9%)
4	Kadioğlu et al., 2001	[27]	Turkey	30	24	5	5/24 (20.8%)
5	Schlegel and Kaufmann, 2004	[28]	USA	NA	31	7	7/31 (22.6%)
6	Çakan and Altuğ, 2004	[29]	Turkey	29	13	3	3/13 (23.1%)
7	Esteves and Glina, 2005	[30]	Brazil	32	17	8	8/17 (47.1%)
8	Gat et al., 2005	[23]	Israel	34	32	18	18/32 (56.3%)
9	Poulakis et al., 2006	[31]	Germany	34	14	7	7/14 (50%)
10	Pasqualotto et al., 2006	[32]	Brazil	30	27	9	9/27 (33.3%)
11	Lee et al., 2007	[33]	Korea	32	19	7	7/19 (36.8%)
12	Ishikawa et al., 2007	[34]	Japan	33.3	6	2	2/6 (33.3%)
13	Cocuzza et al., 2009	[35]	Brazil	29.4	10	3	3/10 (30%)
14	Youssef et al., 2009	[36]	Egypt	31.8	51	14	14/51 (27.5%)
15	Abdel Meguid, 2012	[37]	Egypt	34.9	31	10	10/31 (32.3%)
16	Kiraç et al., 2013	[38]	Turkey	31.7	23	7	7/23 (30.4%)
17	Zampieri et al., 2013	[39]	Italy	33	35	17	17/35 (48.6%)
18	Aboutaleb et al., 2014	[40]	Egypt	29.9	20	6	6/20 (30.0%)
19	D'Andrea et al., 2015	[41]	Italy	37	23	11/	11/23 (47.8%)
20	Shiraishi et al., 2016	[42]	Japan	34	83	20	20/83 (24.1%)
21	Tian et al., 2018	[43]	China	30	130	18	18/130 (13.8%)
22	Zhi et al., 2018	[44]	China	28.1	60	27	27/60 (45%)
23	Sajadi et al., 2019	[45]	Iran	33.8	57	8	8/57 (14%)
24	Elbardisi et al., 2020	[46]	Qatar	35.7	42	11	11/42 (26.2%)
Total					**243**	**831**	**243/831 (29.2%)**

subtypes: 81% for HS, 21% for MA, and 31% for SCO cases. Analysis of this clinical cohort revealed seminal TEX101 AUC of 0.69 (95% CI 0.480.89) with the cutoff of 0.6 ng/m (73% sensitivity, 64% specificity, 70% positive, and 68% negative predictive values). It can be concluded that the higher the TEX101 values, the more likely a favorable histological pattern and, thus, a benefit of varicocelectomy.

Still, this work has some limitations such as the relatively low number of participated NOA cases and the need to have a longer follow-up period.

However, from the abovementioned results, it might be concluded that estimating preoperative seminal TEX101 could be thought of in predicting seminal sperm recovery in NOA cases associated with varicocele after its surgical repair.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

Capsule. Preoperative seminal TEX101 could be considered as a predictor biomarker for sperm recovery for seminal sperm recovery in azoospermic men with palpable varicocele after its surgical repair.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

MWR, TM, HA, MS, and AME designed the study. MWR performed the surgical operation. ZN performed the laboratory analysis and wrote specific sections of the manuscript. TM, MS, and MWR wrote the manuscript. AME and HA revised the final manuscript.

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