Original Article

Serum testosterone levels and other determinants of sperm retrieval in microdissection testicular sperm extraction

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Background: Microdissection testicular sperm extraction (microTESE) has become the standard of care for sperm retrieval in non-obstructive azoospermia (NOA) patients. Understanding the significant determinants of microTESE outcomes may result in improvements in sperm retrieval rates and provide better-informed clinical decisions.

Methods: This is a clinical retrospective study conducted through chart review of 421 NOA patients who underwent microTESE between August 2009 and July 2015 in a tertiary-care referral hospital. Clinical, biochemical and histopathological characteristics were collected. Normal serum testosterone level was defined as testosterone >9.9 nmol/L. Multiple logistic regression was used to identify determinants of microTESE in the studied population. A P<0.05 was considered significant.

Results: Sperms were successfully retrieved in 39.4% of cases. The average testosterone level was 11.51±7.40 and 11.67±6.42 in patients with successful and unsuccessful microTESE, respectively (P=0.820). No significant association was found between serum testosterone level and sperm motility and amount. Of all variables, histological subtype remained to be the most significant determinant of microTESE outcomes in the examined population, with hypospermatogenesis having over a 3-fold higher odd of successful microTESE than sertoli-cell only.

Conclusions: Serum testosterone level appears to have no significant association with microTESE outcomes in NOA. The underlying histological pattern is a significant determinant of the procedure's success.

Keywords: Microdissection testicular sperm extraction (microTESE); TESE; non-obstructive azoospermia (NOA); testosterone; histopathology; sertoli-cell only syndrome

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Introduction

Infertility affects around 15% of couples, of which 20% is accounted for exclusively by male factors (1). Of the limited options that can be offered to non-obstructive azoospermia (NOA) patients (2), microdissection testicular sperm extraction (microTESE) has become the standard of care owing to its superior sperm retrieval rate (SRR), minimal structural-and-functional tissue disruption, and lower post-operative complications (3,4). Unfortunately, there remains a considerable proportion of patients who still fail to have their sperms successfully retrieved with this procedure (2).

As a crucial ingredient of spermatogenesis, testosterone has been a focus of attempts aimed at optimizing the outcomes of microTESE (5,6). Whereas the critical role of testosterone in spermatogenesis has long been established (7), its role in the management of NOA patients is less clear; several reports have investigated the benefit of pre-operative testosterone optimization on microTESE outcomes. While some suggested better outcomes with testosterone optimization (5,8), others showed no effect of testosterone on SRR (6,9,10). The conflicting outcomes of published reports are likely to reflect—among other methodological and demographic variations—the complexity of the body's physiological responses to this hormone, which has been shown to have wide inter-person variations in normal, otherwise healthy individuals (11). In patients with an underlying pathology that prevents the initiation and/or maintenance of normal spermatogenesis, this variation may be more pronounced. With such incongruent findings, further research is needed to better our understanding of the role of testosterone in the operative outcomes of microTESE in NOA. We herein present our experience with microTESE outcomes and its determinants in our population, with particular emphasis on testosterone and its association with the operative outcomes. Our primary objective was to investigate the relationship between pre-operative serum testosterone level and microTESE outcomes. The secondary objective was to identify the significant determinants of the operation's success in our population.

Methods

Study design, population and setting

This is a retrospective medical chart review of NOA patients who underwent microTESE under the care of the Urology Department, King Faisal Specialist Hospital and Research Center (KFSHRC), a tertiary referral center, during the period Aug 2009–July 2015. As described by the World Health Organization guidelines, azoospermia was established by the absence of spermatozoa in two separate analyzed ejaculates (12). Patients with serum testosterone level ≤9.9 nmol/L were considered to have low testosterone. MicroTESE that resulted in the retrieval of at least one sperm was considered positive/successful.

Collected variables

The following variables were collected for each patient: age, body mass index (BMI), serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), total testosterone, estradiol, prolactin, chromosomal aberrations, pre-operative sonographic findings [e.g., testicular volume and varicocele presence (testicular volume was measured using the product of ultrasonographically determined dimensions of the testis, multiplied by 0.52)], previous chemotherapy or bone marrow transplantation, intraoperative sperm retrieval, sperm motility, sperm-freezing amount and post-operative histological diagnosis.

Statistical analysis

Study findings were summarized using frequencies, means and standard deviations. Between-group comparisons were conducted using Student's t- and χ^2 tests, as appropriate. Hierarchical multiple logistic regression was used to assess for potential determinants of microTESE outcomes, to identify the magnitude of each association, and to adjust for potential confounding variables between each of the examined variables and microTESE outcomes. All tests were two-tailed. A P value <0.05 was considered significant.

Ethical considerations

This study was approved by the Institutional Research Advisory Council (RAC), the institutional ethical review board. Informed consent was obtained from each patient.

Results

Sample characteristics

A total of 421 NOA patients underwent microTESE during the identified period. Of those, 371 received no prior treatment with clomiphene or human chorionic gonadotropin (HCG). The characteristics of the study population, along with microTESE outcomes and histopathological findings are shown in *Table 1*.

Testosterone and microTESE success

Out of the 421 operations done, sperms were successfully retrieved in 166 cases (39.4%). There was no significant difference in serum testosterone levels between NOA patients with positive- and negative-microTESE outcomes for whom no prior treatment with clomiphene or HCG

Table 1 Sample characteristics and microTESE outcomes

Variable	Mean ± SD [n] or frequency (%)
Age (years)	36.27±7.09 [421]
BMI (kg/m^2)	30.68±7.19 [418]
Thyroid stimulating hormone (TSH) (mIU/L)	2.9±3.28 [375]
Follicular stimulating hormone (FSH) (IU/L)	17.55±12.81 [410]
Luteinizing hormone (LH) (IU/L)	10.47±6.89 [410]
Prolactin (µg/L)	11.28±10.96 [403]
Estradiol (E_2) (pmol/L)	103.02±67.74 [134]
Testosterone (nmol/L)	11.61±6.82 [404]
Testicular volume (cm^2)	9.48±6.48 [131]
US varicocele (n=170)	
Present on right	32 (18.8)
Present on left	65 (38.5)
Present on right or left	66 (39.3)
Bone marrow transplantation (n=421)	
Yes	18 (4.3)
No	403 (95.7)
Chromosomal analysis (n=59)	
Normal	46 (78.0)
XXY, 47	13 (22.0)
Pre-treatment with clomiphene or B-HCG (n=421)	
Yes	50 (11.9)
No	371 (88.1)
MicroTESE outcome (n=421)	
Positive	166 (39.4)
Negative	255 (60.6)
Histopathology (n=336)	
Sertoli-cell syndrome	240 (71.4)
Hypospermatogenesis	47 (11.2)
Maturation arrest-early	32 (7.6)
Maturation arrest-late	9 (2.1)
Active/normal spermatogenesis	8 (1.9)
Sperm motility (n=163)	
Motile	58 (35.6)
Non-motile	105 (64.4)
Number of straws frozen	3.28±1.797 [165]

microTESE, microdissection testicular sperm extraction; SD, standard deviation; BMI, body mass index; IU, international units; mIU, milli-international units; US, ultrasound; XXY, Klinefelter syndrome karyotype.

was initiated (P=0.599) (*Table 2*). Similarly, the difference in serum testosterone level between positive and negative microTESE groups was not significant when all patients (including those with prior treatment) were examined (P=0.820). The distribution of positive and negative microTESE outcomes was not significantly different between normal (n=223, SRR =38.6%) and low (n=181, SSR =40.3%) testosterone groups (P=0.718); normal and non-treated/naive low (n=131, SRR =42.0%) testosterone groups (P=0.526); or normal and pre-treated low (n=50, SRR =36.0%) testosterone groups (P=0.736).

Testosterone vs. extracted sperm amount and motility

We investigated the relationship of serum testosterone level with the motility and amount of sperms extracted from microTESE. No significant difference was found in sperm motility (P=0.777) or average number of vials that could be frozen after microTESE (P=0.276) between normal and low testosterone groups. Likewise, there was no significant difference between normal and non-treated/naive low testosterone groups in either sperm motility (P=0.987) or average number of frozen straws/vials (P=0.570). The lack of significant difference in sperm motility (P=0.462) and frozen straws/vials (P=0.145) was also applicable to normal *vs.* pre-treated low testosterone groups.

MicroTESE-outcome determinants

In order to identify the potential determinants of microTESE outcomes, we conducted hierarchical multiple logistic regression, in which patients' age and BMI; hormonal profile (FSH and prolactin); and total testosterone and histopathology were entered sequentially in this order. Of all variables, only age (P=0.044) and histopathology (P<0.001) were found to have significant relationship with microTESE outcomes. With each year increase in age, there was an estimated 4% increase in the odds of having a positive microTESE results (aOR =1.039; 95% CI, 1.001–1.077; P=0.044). Hypospermatogenesis was associated with over a 3-fold increase in the odds of having a successful retrieval of sperms than sertoli-cell only syndrome (aOR =4.380; 95% CI, 2.099–9.141; P<0.001). When only naïve NOA were included in the model, only tissue histopathology retained its statistical significance (P<0.001), with hypospermatogenesis conferring around

Table 2 Average total serum testosterone level in successful and unsuccessful microTESE cases

Patient populations	Successful sperm retrieval/ positive microTESE [n]	Unsuccessful sperm retrieval/negative micro TESE [n]	P value (2-tailed)
All cases—mean serum testosterone ± SD (nmol/L)	11.51±7.40 [159]	11.67±6.42 [245]	0.820
Non-treated cases—mean serum testosterone ± SD (nmol/L)	12.24±7.513 [141]	12.63±6.251 [213]	0.599

microTESE, microdissection testicular sperm extraction. SD, standard deviation.

a 4-fold increase in the odds of having a successful sperm retrieval than sertoli-cell only syndrome (aOR =4.961; 95% CI, 2.197–11.202; P<0.001). Of note, testosterone remained a non-significant determinant of microTESE outcomes even after adjusting for all other variables.

Discussion

To our knowledge, this is the first study of microTESE outcomes in relation to testosterone and other factors in our population. In this study, neither serum testosterone level nor FSH was found to be significant predictors of microTESE success. To date, the predictive significance of serum hormonal levels remains controversial (13). While some reports suggested a potential predictive role (10,14-18), the majority found no association between hormonal profiles and outcomes of microTESE (6,13,19-25), which is consistent with the findings reported in this study.

One possible explanation to the lack of relationship between microTESE outcomes and serum testosterone level is the robustness of microTESE itself. Because of the feedback loops, it is expected that derangements in hormonal levels would reflect, at least in part, the extent of the disease within the testicular tissues; the fewer the sperm-production areas that have a normal response, the more extreme the serum hormonal values would be. However, microTESE involves meticulous dissection that makes it resilient to fluctuations in the number of areas of spermatogenesis (26). The active search for islands of spermatogenesis makes sperm retrieval in microTESE less dependent on random chance, which would be otherwise affected by the total number of sperm production areas that are present within the testicular tissue. In other words, a blinded aspiration procedure may be more successful when a higher number of areas with active spermatogenesis are presented within the testicular tissue, but this may be less applicable to microTESE. This may explain, in part, why variations in hormonal levels may not be significantly different between successful and non-successful microTESE procedures. However, such hormonal derangements may affect certain qualities and stages of maturation of the retrieved sperms (e.g., elongated *vs.* round spermatids) and thus the likelihood of fertilization, clinical pregnancies, and live births (27).

Our findings suggest histopathology to be predictive of microTESE outcomes. This is consistent with the previous reports that examined this relationship (10,20,28,29). In fact, to date, histopathology remains the single most significant predictive factor of microTESE sperm retrieval in NOA (13). Of the different histopathological subtypes, sertoli-cell only has been consistently suggested to yield the least microTESE retrieval rates (19). We also found, and as noted by previous reports, the most favorable outcomes to be present in tissues showing hypospermatogenesis (10,19). Unfortunately, histopathology would be of limited practical usefulness as a predictor, since it is determined intra- or post-operatively. Alternatively, performing pre-operative biopsies as a standalone procedure is invasive and may expose the testicular tissue to unnecessary complications (30), especially that multiple biopsies are often needed to reflect the true status of spermatogenesis within the testes (31). Interestingly, higher age at which microTESE was performed was associated with better microTESE outcomes. Data exploring the effect of age on microTESE outcomes are scarce. Bernie *et al.* suggested better chances of microTESE success in older age, but no clear association was identified (13). Albeit counterintuitive, the need for microTESE at an earlier age may reflect a more severe disease, and thus, lower probability of success rates.

Conclusions

Serum testosterone level appears to have no significant association with microTESE outcomes in NOA. Among the clinicopathological patient characteristics, the underlying histological pattern is the most significant

determinant of the procedure's success.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the Institutional Research Advisory Council (RAC), the institutional ethical review board (RAC#2141091). Informed consent was obtained from each patient.

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