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ORIGINAL ARTICLE

Comparison between Open Testicular Biopsy and Testicular Fine Needle Aspiration (TEFNA) Cytology in Obstructive Azoospermia

ZEESHAN AHMAD RAZA[1], ATHAR MAHMOOD[2], HUMAYUN SAEED[3], MUDESSAR RAFAQAT[4], ISMAT ULLAH[5], MUHAMMAD SHAHZAD ANWAR[6]

[1]Medical Officer, [2,3]Assistant Professors, [4]Senior Registrar, [6]Professor & HOD, Department of Urology, Services Institute of Medical Sciences/Services Hospital, Lahore

[5]Consultant Urologist, DHQ Hospital, Narowal

Correspondence to: Mudessar Rafaqat, Email: rafaqatmudessar@yahoo.com, Cell: 0343-6828355

ABSTRACT

Objective: To compare open testicular biopsy and testicular fine needle aspiration (TEFNA) for spermatogenesis in terms of Johnsen's scoring in patients with suspicion of obstructive azoospermia.

Study Design: Comparative study.

Place and Duration of Study: Department of Urology, Services Hospital, Lahore from 24th December 2018 to 23rd December 2019.

Methodology: Forty males with primary infertility and azoospermia (in 3 consecutive reports) were enrolled. Males with normal testicular size and bilaterally palpable vas deference were investigated further regarding their hormone profile (serum FSH, LH, and Testosterone) and scrotal color Doppler ultrasound (CDUS). When hormones and scrotal ultrasound were found normal, appointment was given to patients for procedure, after complete discussion about their diagnosis and plan of management with possible complications. Both procedures; open testicular biopsy and testicular fine needle aspiration (TEFNA) were done simultaneously under local anesthesia. Specimen obtained from both procedures was sent for analysis of spermatogenesis in terms of Johnsen scoring. Patients were discharged after four hours with advice of daily dressing for five days and scrotal support for two weeks and oral Diclofenac Sodium 50mg twice daily after meals for three days.

Results: The mean values of Johnsen scores were 7.7±2.8 in open biopsy while in TEFNA 9.9±0.95 and statistically significant (<0.05) results were found. Among 40 patients, 33(82.5%) patients showed spermatogenesis on open biopsy and 39(97.5%) on TEFNA. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and accuracy of TEFNA in diagnosing spermatogenesis was 100%, 14.2%, 84.6, 100.0% and 85.0% respectively.

Conclusion: Testicular fine needle aspiration is found to be a simple, reliable, and least invasive mode of diagnosing and management of azoospermia in adult males with minimal complications.

Keywords: Open testicular biopsy, TEFNA, Infertility, Johnsen score

INTRODUCTION

Infertility is defined as the failure to achieve a clinical pregnancy after 12 months or more of regular, unprotected sexual intercourse. [1] The overall incidence of infertility is 15%. The male factor contributes 30%, the female factor contributes 30%, and both contribute 30%, while the remaining 10% of the causes cannot be identified.[2]

Azoospermia, the absence of sperm in the ejaculate, is found in approximately 1% of all males and 10-15% of infertile males. Causes of azoospermia may be pre-testicular, testicular, or post-testicular pathologies. Post-testicular causes of azoospermia account for about 15-20% of cases, in which spermatogenesis is normal but sperm are not present in ejaculate.[3] Physical obstruction of the post-testicular genital tract at different levels is considered to be the cause of azoospermia. [4]

Assessment of spermatogenesis in the testis is the next step in management if testicular size and hormone (follicle stimulating hormone) are normal and the diagnosis is heading towards obstructive azoospermia. [5]

By open testicular biopsy, assessment of spermatogenesis is done by obtaining a piece of testicular tissue from a single part of the testis. [6] If mature spermatozoa are visible (Johnsen's score is above 9), azoospermia is obstructive. In the absence of mature spermatozoa, azoospermia is non-obstructive. [7]

Infertility management is revolutionised by assisted reproductive techniques (ART). Conformation of spermatogenesis and sperm retrieval can now be done with a variety of newly invented procedures. Percutaneous testicular sperm aspiration (TESA), percutaneous epididymal sperm aspiration (PESA), microsurgical epididymal sperm aspiration (MESA), testicular sperm extraction (TESE), micro-dissection testicular sperm extraction (m-TESE), and testicular fine needle aspiration are commonly done procedures. [8]

It is considered that spermatogenesis is not uniform in the whole testis. [9] An open testicular biopsy is a procedure in which small pieces of seminiferous tubules are taken from the testis. If no sperm are found on the Johnsen scoring and the sample is taken from only one part of the testis, spermatogenesis is considered absent in the entire operated testis.Testicular fine needle aspiration cytology, on the other hand, is a technique for obtaining seminiferous tubule samples from various parts of the testis.[10]

The proposed study was designed to compare open testicular biopsy with testicular fine aspiration cytology done simultaneously in the same patient under local anaesthesia for confirmation of spermatogenesis by Johnsen scoring. So far, no such diagnostic study has been carried out in Pakistan. The results may have diagnostic and therapeutic value in patients with obstructive azoospermia.

MATERIALS AND METHODS

This comparative study was conducted Department of Urology, Services hospital Lahore from from 24th December 2018 to 23rd December 2019 and 40 males were enrolled. All males age 20 years and above, primary infertility and suspicion of obstructive azoospermia were included. Abnormal FSH, LH, testosterone, decreased testicular size on Doppler ultrasound and history of genital tuberculosis, epididymo-orchitis were excluded. Detailed history was taken and physical examination was carried out including examination of external genitalia. Amongst them, men with normal testicular size and bilaterally palpable vas deference were investigated further regarding their hormone profile (FSH, LH, and Testosterone) and scrotal color Doppler ultrasound (CDUS). After selection as per criteria, complete information was provided to the patients about their problem. Procedure details, possible outcome and complications were explained to them. Appointment for procedure was given to patient and consent was taken in writing. The procedures were performed as a day case surgery. It was performed in supine position. Intravenous line of the patient was secured with 18 Fr cannula. Cardiac monitor and pulse oxy-meter was attached, for vital signs monitoring and Oxygen saturation measurement throughout the procedure. After adequate

exposure from umbilicus to the mid-thigh, the patient was draped with povidone-iodine solution.

Procedure was performed under local anesthesia (Spermatic cord block). Spermatic cord was palpated and immobilized in the left hand between thumb and index finger. Needle of disposable syringe filled with 2% injection lignocaine was inserted vertically in the spermatic cord. About 3ml of lignocaine was injected in the spermatic cord. Additional 2 ml was injected by tilting syringe obliquely to spread the anesthetic effect thoroughly in spermatic cord. Additionally 1 ml of 2% lignocaine was applied at the site of incision of open testicular biopsy. Then assistant hold the left testis in such a fashion that skin over anterior surface of testis was stretched, while epididymis stayed posterior. Transverse scrotal incision of about 1 cm was made on to the scrotal skin at mid testis. After securing hemostasis, facial layers were incised and tunica vaginalis was opened. About 5 mm incision was applied on tunica albuginea to expose the seminiferous tubules. Sample of seminiferous tubules about the size of rice grain was dissected out with a pair of iris scissors, and immediately was preserved in the Bouin solution. For testicular fine needle aspiration cytology, three specimens of seminiferous tubules were collected from upper, middle and lower parts of testis. First specimen was obtained through the biopsy incision with the 21 Fr butterfly needle which was attached with 20ml disposable syringe filled with media (sperm wash media with hepes buffer) and tubing of the butterfly needle was rinsed with media. Butterfly needle was inserted vertically in rotatory moments in the testis with suction generated manually by pulling plunger of the syringe till part of seminiferous tubules (about the size of rice grain) was visible in the tubing of butterfly set. Specimen was preserved in a container filled with bouin solution and was considered as biopsy from middle part of testis. Similar specimen was collected from upper and lower parts of testis and was preserved in separate containers filled with Bouin solution. The tunica albuginea was stitched with polygalactin 4/0 continuous sutures and wound was closed in layers. Skin was closed with polygalactin 4/0 interrupted fashion. Dressing and scrotal elevation was applied. Patient was kept under observation in ward for next 4 hours and then was discharged on oral Diclofenac Sodium 50mg for pain. Four specimen of seminiferous tubules obtained from each patient were labeled properly. Those separately preserved specimen were transported to Histopathology Department with in half hour by laboratory technician. Histopathology request form with particulars of patient, history, scrotal examination finding and investigations were sent along with specimen for evaluation of Johnsen scorings for spermatogenesis.

Patient was discharged after four hours with advice of daily dressing and oral Diclofenac Sodium 50mg twice daily after meals for three days. Patient was advised to apply, daily dressing for five days, and scrotal support for two weeks. First follow up visit was on 7th post-operative day. In follow up, history of the patient was taken and physical examination of operated area was done for any possible complications for example, wound infection or scrotal swelling etc. and were managed accordingly. Second follow up visit of the patient was on 14th post-operative day with reports of Johnsen's Scoring.

The data were entered and analyzed using SPSS-25.0. Comparison was made between Johnsen's scoring of open testicular biopsy tissue and tissue obtained by TEFNA. The Johnsen score was compared with independent sample t-test between testicular biopsy and testicular fine needle aspiration. A p-value of less than or equal to 0.05 was considered as significant. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were evaluated using following formulas.

RESULTS

In this study, ages were categorized in two groups; one is 20-35 years age group and second is 36-50 years age group. There were 14 (35.0%) patients in 20-35 years age group, while 26(65.0%) in 36-50 years age group. When Johnsen score was compared in both techniques, difference was found statistically significant. In open biopsy, the mean value of Johnsen score was 7.7±2.8 with minimum value as 2 and maximum as 10 whereas in TEFNA, the mean value of Johnsen score was 9.9±0.95 with minimum value as 4 and maximum value as 10. Among 40 patients, 33(82.5%) patients showed spermatogenesis on open biopsy and 39(97.5%) on TEFNA. When t-test applied between techniques to compare the Johnsen's score, statistically significant difference ($P<0.05$) was seen. Johnsen's score value was better in TEFNA group ($p=0.0001$). Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of TEFNA in diagnosing spermatogenesis was 100%, 14.2%, 84.6, 100.0% and 85.0% respectively. Only one (2.5%) patient had wound infection, healed with antibiotics given for five days and one had scrotal swelling, which settled with scrotal elevation (Tables 1-2).

Table 1: Demographic Information of the Patients (n=40)

Variable	No.	%
Age (years)		
20-35	14	35.0
36-50	26	65.0
Wound Infection		
Yes	1	2.5
No	39	97.5
Scrotal Swelling		
Yes	1	2.5
No	39	97.5
Spermatogenesis by open biopsy		
Yes	33	82.5
No	7	17.5
Spermatogenesis by TEFNA		
Yes	39	97.5
No	1	2.5

Table 2: Presence of Spermatogenesis by Open Biopsy Versus TEFNA

Spermatogenesis by TEFNA	Spermatogenesis by open biopsy		Total
	Yes	No	
Yes	33	6	39
No	0	1	1
Total	33	7	40

Sensitivity = 100% Specificity = 14.3%
Positive predictive value = 84.6%
Negative predictive value = 84.6%100%
Accuracy = 85% Prevalence = 82.5%

DISCUSSION

This is a modern study in diagnostic work up of male infertility, which enhances the chances of detection of spermatogenesis in suspected cases of obstructive azoospermia. Very limited research has been done on minimal invasive diagnostic work up for azoospermia in Pakistan.

In our clinical set up, open testicular biopsy is still considered as the gold standard method for diagnosis of male infertility. Testicular FNAC has been introduced very recently[11,12] who demonstrated superior accuracy of cytological diagnosis with histological categories in non-obstructive azoospermia. Additionally testicular FNAC is a minimally invasive technique in diagnosis and management of azoospermia.

Micro-assisted fertilization procedures are of tremendous benefit to infertile couples in the present era of in-vitro fertilization, as the only need for these techniques is a viable sperm and ovum. Therefore report of presence or absence of mature sperm in cytological smears is sufficient.[13]

The findings of our study, in which we compared testicular FNAC and open testicular biopsy in infertile males is in consistent with different studies conducted before, as we compared these findings with previous studies. The mean age of males described in several researches is as 27 years[14], 34 years[15], 36 years[4], 27 years[7] and in the present study is 35.90 years.

Dajani and Kilani[16] conducted a study to evaluate the use of testicular FNAC by grading the cytological smears in thousands infertile men and observed that the grade-C which indicates sertoli cell only syndrome. Our study found that the grade-B was the commonest grade.

Mourad et al[17] found the pattern of testicular pathology in male infertility and ranked the testicular biopsies as per "Johnsen scoring system". Predominant Johnsen score in the study was 2 indicating SCOS.

Arango et al[18] conducted the first well-designed study regarding the sperm retrieval rate (SRR) by TESA versus TESE in a cohort of 37 men with non-obstructive azoospermia. Our study also highlighted superior spermatogenesis detection with TAFNA as compared to OTB on basis of Johnsen scoring.

Nonetheless, several observational studies have found that these two techniques, testicular FNAC and open testicular biopsy, have comparable SRR. Mourad et al[17] performed TESA on 85 azoospermic males, with an average of 15 punctures for aspiration in each testis, and found mature testicular spermatozoa in 65 (58.5%) of the patients. Our method of needle aspiration of whole testicular tubule, not only detected spermatogenesis but also grade their maturity analysis on basis of Johnson scoring.

In a big research, mature sperm were found in 63 percent of TESA procedures performed using a 21-G needle.[19] In this study, it was discovered that TESA had a substantially lower SRR than TESE on average. In subgroups of individuals with serum FSH >15 IU/L and testicular histology indicating hypospermatogenesis, however, the SRR was equivalent between TESA and TESE.

Blind biopsy can be replaced by FNAC-guided TESE. There are several limitations, such as the fact that FNAC does not give testes architectural information. It does not include information on tubular basement membrane thickness, tubular diameter, or interstitial tissue condition. Histology can help detect azoospermia-causing conditions including atrophy, fibrosis, and Leydig cell hyperplasia. But through FNA, above mentioned parameters are difficult to measure.[20]

Some individuals have persistent discomfort, which can be alleviated with scrotal support and medications. Additionally there are documented evidences that patients may develop neurogenic shock during TEFNA. When a thick needle (20G) is used, hematoma development is likely.[9]

Although TEFNA is simple, cost-effective and minimally invasive procedure, but its SRR is less as compared the standard method of micro-dissection TESE. This is the major reason behind the limited use of TEFNA for sperm retrieval in reproductive medicine.[5]

The association of different cell indices with cytological diagnosis revealed an increase in Spermatic index (SI) and a reduction in Sertoli index (SEI) in normal spermatogenesis, whereas hypospermatogenesis and maturation arrest exhibited a gradual decline in SI and an increase in SEI. In hypospermatogenesis, SEI was shown to be higher than in maturation arrest. SI and SEI were found to be zero in SCOS. Open biopsy was determined to have an overall accuracy of 82.5 percent, while TEFNA was found 100% accurate in diagnosing normal spermatogenesis.

The mean value of Johnsen score in open biopsy was 7.7±2.8 with minimum value as 2 and maximum as 10 whereas in TEFNA, the mean value of Johnsen score was 9.9±0.94 with minimum value as 4 and maximum as 10. When applied t-test in both techniques to compare the Johnsen score significance the results find significant as the p value is less than alpha. Johnsen's score value is better in TEFNA group. While comparing both procedures TEFNA and OTB, no third procedure was considered as gold standard. To overcome this deficiency, a fair agreement is set on the basis of Cohen's Kappa coefficient, which is used to estimate interrater reliability, usually applied in context of test-retest. The range of kappa is from -1 to +1. Kappa statistics when applied to this study, result was 0.21. This reading showed significant reliability of results obtained in this study.

The results in our study highlighted that TEFNA covered wider area of testicular tissue sampling and tubules were taken from different levels within testicular architecture. This maximized chances of spermatogenesis when assessed in three different tissue sample taken from three different sites whereas, single sample was taken from only one peripheral part of testis in open testicular biopsy, which relatively reduces their chances of spermatogenesis detection.

This study conducted on infertile men with suspicion of obstructive azoospermia, majority of comparative analysis done in past, were in patients with non-obstructive azoospermia and were mostly for sperms retrieval only. Another most distinguished feature of the study was that it not only detect presence of spermatogenesis but it also assessed the maturity of sperms on basis of sperms maturation standardized tool Johnsen scoring. Moreover, the technique of TEFNA is least invasive and is more informative about the spermatogenesis in major area of testes. Complications are rare after TEFNA.

CONCLUSION

Testicular fine needle aspiration is found to be a simple, reliable, and least invasive mode of diagnosing and confirming the presence of mature sperms and minimal complications in adult males with obstructive azoospermia.

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