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Fine Needle Aspiration Cytology (FNAC) Of Testis versus Open Biopsy in Cases With Azoospermia.

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ABSTRACT

Background: Aims: The objective of this study was to assess the diagnostic accuracy and cytological features of fine needle aspiration cytology smears (FNAC) in determining the causes of azoospermia as compared to open testicular biopsy. **Methods:** 35 patients with non-obstructive azoospermia were subjected to the study; the age of them was 20-40 years. Each patient was subjected to the taking history and clinical examination. Laboratory tests were done including urine analysis, three consecutive semen samples and endocrine evaluation. Then, cord block was achieved with 1% lignocaine; and fine needle aspiration was be done, then staining the smears with Leishman or hematoxylene and eosin stains. In the same sitting, testicular biopsy was taken, then Hematoxylin & Eosin (H&E)-stained sections were prepared. Histopathological and cytological evaluation was investigated; and photographs were be taken. Then, statistical analysis was performed. **Results:** The concordance of FNAC with open biopsy in the diagnosis of azoospermia was 100%. FNAC smears was associated with fewer complications such as bleeding, pain and hematoma than in case of open biopsy. **Conclusion:** The technique of testicular FNAC is simple, more than one specimen could be taken safely. FNAC is representative and correlates well with testicular biopsy histology.

Keywords: Fine needle aspiration cytology, azoospermia, testicular biopsy, testicular cytology.

INTRODUCTION

Since many years past, the wife has always been blamed for infertility; the possibility of treatment of the male for infertility is a recent one. Early in the beginning of the twenty century it is failed to find sperms in a post coital test.[1] This finding raised the possibility that husband could be responsible for infertility. The available statistics showed that the male factor is responsible for about 40% to 50% of all cases of sterility.[2]

Azoospermia is present in about 10-15% of men evaluated for infertility. The case might occur with different testicular alterations.[3,4]

Open biopsy has proven to be an important procedure to clarify the pathogenesis of male infertility and to determine the prognosis. However this method is invasive and traumatic; and the risk increases when done on both sides. Therefore needle puncture has been proposed as an alternative method.[5]

FNAC was first described early in 1913.1 However, utilization of FNAC of the human infertile testes was described in1971.[6] but the morphological features of cells obtained was not fully described. Later on; cytological features of the seminiferous tubules were described by other authors.[7-9] FNAC testes did not take popularity because of limited awareness about usefulness of the technique, lake of expertise in aspiration and interpretation of the cytological variations, and the paucity of information about architectural details of cytology. The current study was to assess the diagnostic accuracy and cytological features of FNAC in determining the causes of azoospermia as compared to open testicular biopsy.

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MATERIALS AND METHODS

Thirty-five patients with non-obstructive azoospermia were subjected to the study after written concepts. The age group of them was 20-40 years, and selected from those attending October 6 University Outpatient Clinic of Dermatology &

Andrology, during the period from 25th of November 2015 to the 1st of September 2016. These patients will be subjected to bilateral testicular fine needle aspiration (FNAC) for cytological evaluation as well as bilateral open testicular biopsies for histopathological correlation procedures. Three of these patients included in the total; had single testis. Therefore, we examined 35 patients, 67 samples (32 patients, biopsy from bilateral testis, i.e. 64samples, in addition to 3 patients with single testis, i.e., 3 samples). We added two patients out of the total, one patient with bilateral absence of the vas and the other patient, previously diagnosed clinically as epididymo-orchitis. We utilized the case of absence of vas deference as a control for normal spermatogenesis, and the case of epididymo-orchitis as a control for inflammatory conditions.

Each patient was subjected to history taking including; history of drug intake e.g. hormones, antidepressants, antacids, sulphathiazine…etc., duration of infertility and systemic disease. Clinical examination was done for each patient with special emphasis on genitalia (penis, testis size & consistency, inguinal regions, presence or absence of hypospadias). Laboratory tests including urine analysis and endocrine evaluation were performed on all patients. Three consecutive semen samples: We collect three consecutive samples within 2 weeks, each sample after 3-4 days sexual abstinence period.

FNAC was done using 23-gauge 1.5 inch needle, after cord block with 1% lignocaine.. Smears were made on four slides after cleaning with 95% alcohol and drying in air. The smears then fixed by placing in 95% alcohol for at least 20 minutes. Then staining with Leishman or hematoxylene and Eosin stains. In the same sitting, testicular biopsy was taken, then fixed immediately in Bouin` fixative, routinely processed to form paraffin blocks. Then H & E-stained sections were prepared.

Histopathological and cytological evaluation and photographs will be taken using Binuclear Light Microscope (Olycmpus CX 31) with digital camera model (Color-Cmos Camera, 5XY-150).

At first, we examined H&E slides of biopsy, then comparing with the FNAC smear slides. Light Microscopy examination: at first we examined biopsy slides in order to diagnose the specimens. In the comparison of FNAC smear with the biopsy, we began with that cases, diagnosed by biopsy as Sertoli cell only syndrome, with little or no fibrosis or hylinosis of the tubules, rarefaction of the interstium and little Lyedig cells, in order to find Sertoli cells only in the smears. Then we examined FNAC smears of patients diagnosed by biopsy as complete arrest of spermatogenesis at Secondary and/or primary spermatocytes, with rarefaction of the interstitium and little Lyedig cells, in order to find secondary and primary spermatocytes in the smears. Then we examined FNAC smears from patients diagnosed by biopsy as complete arrest of spermatogenesis at the level of spermatids, in order to find spermatids in the smears. Then we examined, FNAC smears from the patient with absence of vas deference (obstruction of spermatogenesis), to find sperms clearly. Then FNAC smears from cases of incomplete maturation arrest, hypospermatogenesis (mild, moderate and severe) were examined, to find any of sperms. Lastly we examined FNAC smears taken from patients diagnosed by biopsy as complete fibrosis and hyalinosis, to find fibrous tissue, fibroblasts. The statistical analysis was performed using (SPSS 16.0 for Windows; SPSS Inc. Chicago, Illinois, USA).

RESULTS

The range of age of the 35 patients was from 22 to 38 years old with mean 28.51 ± 4.47, and median 28. Twenty-five patients (71.4%) with age ranging from: 22-30 years. Ten patients (28.6%) with age ranging from 31-38 year. The duration range of infertility was from 2 to 6 years.

In regards to the laboratory tests; Fructose test was positive in 27 cases (77.1%), LH IU/l: (Mean ± SD 6.27 ± 1.27), Median (Range) 6.32 (3.24 – 8.50). FSH IU/l: Mean ± SD 12.49 ± 4.30 Median (Range) 13 (3 – 20). Free Testosterone ng/ml: Mean ± SD 4.07 ± 1.75 Median (Range) 3.93 (1.80 – 7.50). Total Testosterone ng/ml: Mean ± SD 13.5 ± 3.92 Median (Range) 12.50 (8 – 21). Prolactin ng/mL: Mean ± SD 14.97 ± 2.49 Median (Range) 14.60 (7.68 – 23).

Microscopic examination of the biopsy showed; Normal spermatogenesis in 4 cases (16%), Obstructive spermatogenesis in 12 cases (17.9%), Hypospermatogenesis in 12 cases (17.9%); mild hypospermatogenesis in 4 cases, moderate hypospermatogenesis in 6 cases, severe hypospermatogenesis in 2 cases, Incomplete maturation arrest in one case(1.5%), complete maturation arrest in 14 cases (20.9%), Sertoli cell only syndrome (germ cell aplasia) in 23 cases (34.3%); without stromal fibrosis in 6 cases, with stromal fibrosis only in 10 cases, with stromal fibrosis and Lyedig cell hyperplasia in 5 cases, Testicular infarction in one case (1.5%).

We began cytological examination by that patients with diagnostic biopsy (Sertoli cell only syndrome). Sertoli cells appeared as rounded to oval cells with thin cytoplasm. The nuclei were dark, granular or clear with prominent nucleoli. The cells may be elongated, tall or oval with central folding. The slide smear of that patients with complete arrest at secondary and/or primary spermatocytes showed both types of cells. Primary spermatocytes appeared as large rounded cells with abundant esinoplilic or basophilic cytoplasm. The nuclei were granular. Secondary spermatocytes appeared relatively

smaller, with little cytoplasm, dark nuclei and unapparent nucleoli. The slide smear of that patients with complete arrest at spermatids showed spermatids, in addition to spermatocytes and Sertoli cells. Spermatids appear as small rounded nuclei (contain haploid no. of chromosomes), without tails, or ghosts of tails. The slide smear of that patient with absence of vas showed plenty of sperms.

Table 1: Cconcordance between cytological findings and biopsy findings (100% concordance in the main diagnosis)

Histopathological finding	Subtypes	Cytological finding
Normal spermatogenesis		Normal spermatogenesis
Obstructive spermatogenesis		Normal spermatogenesis
Hypospermatogenesis	Mild	Hypospermatogenesis
	Moderate	Hypospermatogenesis
	Severe	Hypospermatogenesis
Incomplete maturation arrest		Incomplete maturation arrest
Complete Maturation arrest	At spermatids	Complete maturation arrest at spermatids.
	At secondary and/or primary spermatocytes	Complete maturation arrest at primary, secondary spermatocytes
Sertoli cell only	Without stromal fibrosis	Sertoli cell only syndrome
	With stromal fibrosis only	Sertoli cell only syndrome
	With stromal fibrosis and Lyedig cell hyperplasia	Sertoli cell only syndrome
Testicular infarction		Testicular infarction
Total	67(100%)	67 (100%)

Table (2): Comparison of Fine needle aspiration cytology versus open biopsy histopathology as regards complications.

Complications	FNA cytology (N=67)		Open biopsy histopathology (N=67)		Test ‡	p-value (Sig.)
	No.	%	No.	%		
Testicular bleeding						
Absent	60	89.6 %	2	3%	56.017	<0.001 (HS)
Present	7	10.4 %	65	97%		
Hematoma formation						
Absent	62	92.5 %	58	86.6 %		
Present	62	92.5 %	58	13.4 %		
Orchitis						
Absent	67	100 %	65	97%		
Present	0	0%	2	3%		

- ‡ McNemar's test.
- p< 0.05 is significant.
- Sig.: Significance.

Table 3: Comparison between Fine needle aspiration cytology and open biopsy histopathology as regards time and cost.

Time and cost	FNA cytology (N=67)	Open biopsy histopathology (N=67)	Test	p-value (Sig.)
Time	(min.)	(hours)		
Mean ± SD	12.07 ± 2.65	23.14 ± 9.13	-7.145	<0.001 (HS)
Median (Range)	11 (5 – 19)	20 (15 – 50)		
Procedure cost (EP)				
Mean ± SD	60 ± 0	1200 ± 0	-8.185	<0.001 (HS)
Median (Range)	60	1200		

- Wilcoxon singed ranks test.
- p< 0.05 is significant.
- Sig.: Significance.
- EP : Egyptian pound

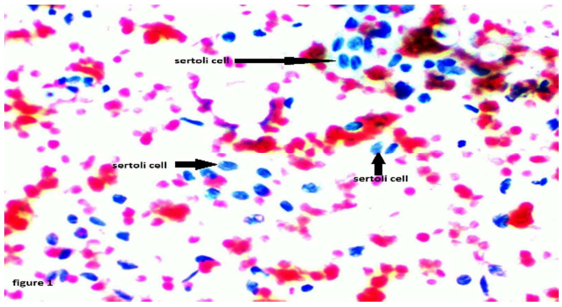

Figure 1: Fine needle aspiration cytology FNAC smear from Rt. testis of the same case of Sertoli cell only syndrome, showing Sertoli cells, X400.

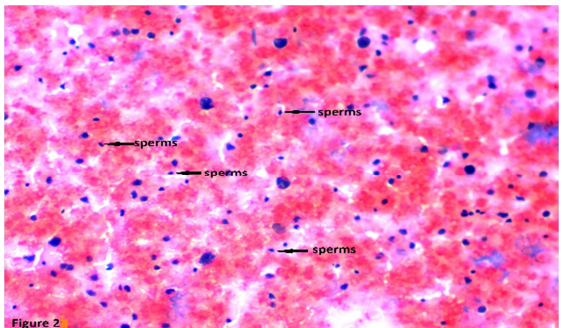

Figure 2: fine needle aspiration cytology FNAC Smear from the same case of absence of vas deference showing (Mature Sperms).

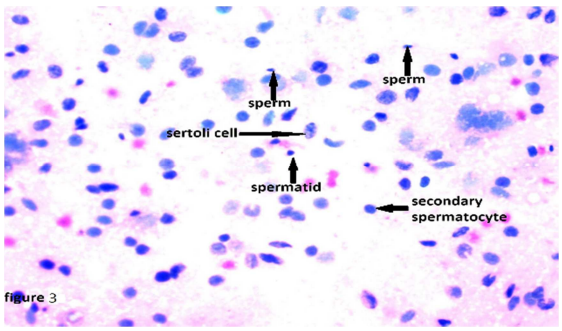

Figure 3: Fine needle aspiration cytology (FNAC) smear showing a case of obstruction of spermatogenesis, showing mature sperms, spermatids, and secondary spermatocytes.

The sperms appeared as small rounded to oval nuclei with hardly seen thin tails. The fibroblasts appeared as long tapering cells with long tapering nuclei [Figures 1-3].

In regards concordance between FNAC and biopsy examination; we found 100% concordance in the main diagnosis. However, in FNAC; we cannot differentiate the subtypes of normal spermatogenesis (normal spermatogenesis/obstruction spermatogenesis), Hypospermatogenesis (mild, moderate or severe subtypes), Sertoli cell only syndrome (with stromal fibrosis, stromal fibrosis only or without stromal fibrosis) [Table 1].

FNAC underwent less complications than testicular biopsy; 7cases only underwent bleeding in contrast to 65 cases bleed with biopsy, 5 cases only underwent testicular hematoma in contrast to 9 cases with biopsy, while orchitis did not occur with FNAC, it occurred in 2 cases with biopsy [Table2].

In regard to time of the procedure and cost; FNAC took about 11-12 minutes while biopsy took 20-23 hours. The procedure cost was about 60 Egyptian pound for FNAC, while 1200 for biopsy [Table 3].

DISCUSSION & CONCLUSION

In our study; we can distinguish obstructive spermatogenesis from normal spermatogenesis on the basis of biopsy examination. However, we cannot distinguish them on the basis of cytological examination. The diagnosis depends on the architectural features of seminiferous tubules in obstructive spermatogenesis,[10] which do not appear in FNAC smear. On examination of FNAC smears, we can identify hypospermatogenesis, with few sperms. Histologically, we can distinguish the subtypes of mild, moderate and severe hypospermatogenesis, according to some authors.[11] These subtypes cannot be evaluated by cytology. One case of incomplete maturation arrest (hardly seen few sperms) can be evaluated by biopsy and cytology, this was a surprise for us. This may be attributed to just good chance of FNA that attack the mature seminiferous tubule. However this result was an exception. Complete maturation arrest at the level of spermatids and at the level of secondary and/or primary spermatocytes can be identified by biopsy and cytology as well. The same result was found by other authors.[12] Sertoli cell only syndrome could be evaluated clearly by biopsy and cytology, but if there`s extensive fibrosis, Lyedig cell hyperplasia, cytology could not be able to clarify these details. The concordance between FNAC and biopsy was 100% in this study, especially in the main diagnosis, but however, some details that depend on the architectural features could not be evaluated by FNAC smears. However, FNAC take a great advantage over biopsy in evaluation of spermatogenesis in the azoospermic testses, especially in NO A. because it conserve tissues.[13] The finding could also be seen by an others.[14-16] One case of testicular infarction could be easily evaluated by biopsy and cytology.

Our study showed that FNAC was associated with fewer complications than open biopsy. Testicular bleeding was absent in 60 out of 67 specimens obtained by FNAC by (89.6%). Bleeding was presented only in 7 out of 67 by (10.4%). On the contrary, bleeding was presented in 65 open biopsies out of 67 by 97%. In a series of 34 cases, it was reported that open testis biopsies resulting in signs of intratesticular bleeding was 29% (10 of 34). 17 Thus it appears that the needle biopsy is associated with fewer complications than the open biopsy, this was in consistent with study of 64 patients.18 Sixty four patients were evaluated after open testicular biopsy for non-obstructive azoospermia with serial scrotal sonography, histological analyses and evaluation of the success of repeated sperm retrieval attempts. Three months following open biopsy, patients complained of ultrasonagraphic abnormalities by (82%). Abnormalities in the testis suggesting resolving inflammation or hematoma at the biopsy site. By 6 months, the acute changes had resolved leaving linear scars or calcifications.[18]

Some authors reported that the open excisional biopsy technique is an invasive procedure which may cause discomfort for the patient, even if only taken through a small incision and even after meticulous homeostasis. Percutaneous puncture of the testis using a fine needle is a less invasive procedure which has been used successfully to recover testicular samples for diagnostic purposes.[19]

Another retrospective study, showed that patients undergoing percutaneous sperm aspiration tended to report less pain and discomfort once at home than those who had had an open biopsy.[20]

Based on the results, we could conclude that the technique of testicular fine needle aspiration cytology is simple, more than 1 specimen can be taken safely. Testis fine needle aspiration cytology (FNAC) correlates well with testis biopsy histology in the evaluation of male infertility. Complications in fine needle aspiration cytology (FNAC) are less than in the open biopsy, as it is minimally invasive. Fine needle aspiration cytology (FNAC) could be done in out-patient clinic, because it's not in need for general anesthesia or operative room. Fine needle aspiration cytology (FNAC) technique is not time consuming, comparing to open biopsy. Materials and the preparations for the specimens of fine needle aspiration cytology (FNAC) are cost effective less than open biopsy.

We recommend to perform FNAC of the testis instead of the invasive biopsy, as a simple, rapid, non-invasive method, with informative data in evaluation of spermatogenesis in the infertile testes.

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