Testicular fine needle aspiration cytology in azoospermic males

RC Adhikari

Department of Pathology, Tribhuvan University Teaching Hospital, Maharajgunj, Kathmandu, Nepal

Corresponding author: Dr. Ram Chandra Adhikari, MD, Associate Professor, Consultant Pathologist, Department of Pathology, Om Hospital and Research Centre, Chabhil, Kathmandu, Nepal, GPO Box no. 2496, e-mail: rcadhikari@hotmail.com

ABSTRACT

One hundred one azoospermic males were subjected to fine needle aspiration cytology (FNAC) of the testis and 11 of them underwent testicular biopsy as well. In 99 cases, the aspiration was adequate for classifying into different categories, which are as follows: Sertoli cell only syndrome (46), normal spermatogenesis (27), hypospermatogenesis (10), tubular / peritubular sclerosis (10) and maturation arrest (6). The percentage population of Sertoli cells and spermatogenetic cells and cell indices including spermatic index, Sertoli cell index and sperm – Sertoli cell index were calculated. In normal spermatogenesis, fine needle aspiration (FNA) smears showed upto 40.0% Sertoli cells and spermatozoa were the predominant spermatogenetic cell type. In Sertoli cell only syndrome, there were sheets of Sertoli cells that constituted 88.0–100.0%. Progressively increasing values of the Sertoli cell index and progressively decreasing values of the sperm – Sertoli index were seen in normal spermatogenesis, maturation arrest, hypospermatogenesis, tubular / peritubular sclerosis and Sertoli cell only syndrome. In this study, complete agreement between testicular FNAC and histology was noted. Pecentage cell counts and cell indices in testicular FNA and 100.0% cyto – histo correlation make the FNAC preferable to biopsy in assessing azoospermic males.

Keywords: Testis, azoospermia, FNAC.

INTRODUCTION

Azoospermia is present in about 10.0–15.0% of men evaluated for infertility.[1] This condition represents the different testicular alterations including normal spermatogenesis, hypospermatogenesis, maturation arrest, Sertoli cell only syndrome and tubular / peritubular sclerosis.

In the assessment of male fertility, semen analysis and histological examination of testicular biopsy are the most accepted methods. Testicular biopsy has been indicated to investigate seminiferous tubule function since the 19th century and was used clinically by Hotchkiss.[2] Since then, various less invasive methods for obtaining testicular material have been evaluated.

In 1965 and 1971, Obrant and Persson[3] and Persson *et al*.[4] Described the fine needle aspiration cytology (FNAC) method for obtaining material for cytological evaluation of spermatogenesis. The cytological features of seminiferous epithelium was described in detail by Schenck and Schill.[5] Compared with testicular biopsy, which is more invasive and painful, and requires theatre facilities, FNA is a more rapid and minimally invasive.

This study was conducted to determine the diagnostic and clinical value of FNAC in azoospermic males and evaluate the value of percentage cell counts and cell indices of testicular FNA and correlate with histological categories.

MATERIALS AND METHODS

This study was retrospective analysis of FNAC of testis from the record of department of Pathology, Om Hospital and Research Centre, Chabhil, Kathmandu, Nepal between January 2006 and December 2008. During this period of 3 years, 101 patients were referred for FNAC of testis to the Department of Pathology. All patients were infertile and their semen analysis revealed azoospermia on at least two occasions, 2 weeks apart.

FNA of testis was done after explaining the procedure and possible complications. Aspiration was performed on one testis under sterile conditions. The FNA was performed under local anaesthetic (2% lignocaine) infiltrate into the cord structures and scrotum locally. 21 gauze needle attached to a 10 ml disposable syringe was used for FNA. Aspirate usually consisted of thread like material, mixed with blood. The smears were air dried, fixed in methanol, and stained by Giemsa.

The aspirate was considered to be adequate when at least 2000 cells or 100 clusters of 20 cells each were obtained.[7] The cells were classified according to the morphologic criteria of Schenck and Schill[5] and were divided into 5 categories: normal spermatogenesis, Sertoli cell only syndrome, hypospermatogenesis, maturation arrest and tubular / peritubular sclerosis.[8]

The percentage populations of Sertoli cells and various spermatogenetic cells, including spermatogonia, primary

Table-1: Cytological diagnosis in 99 cases

Category	No. of cases (%)
Normal spermatogenesis	27 (27.3)
Sertoli cell only syndrome	46 (46.4)
Hypospermatogenesis	10 (10.1)
Maturation arrest	6 (6.1)
Tubular / peritubular sclerosis	10 (10.1)
Total	**99 (100.0)**

spermatocytes, spermatids, and spermatozoa, were calculated. Different cells in FNA smears were recognized by their nuclear and cytoplasmic characteristics.[5,9] All the counts and indices were expressed as percentages. The spermatic index was calculated as the ratio of mature spermatozoa to total spermatogenetic cells. The Sertoli cell index was given by the ratio of Sertoli cell to all spermatogenetic cells. The sperm – Sertoli cell index was the ratio of spermatozoa to Sertoli cells.[10]

Cytology and histology correlation was done in 11 cases.

Values have been reported as mean with range. Comparison of mean value was done and one sample t-test was applied.

RESULTS

The mean age of these men was 30.9 years with a range of 21 to 51 years. The testicular aspirate was adequate for opinion in 99 cases out of 101 cases. The cytological diagnosis in aspirate from 99 cases was depicted in Table-1.

In all of 27 cases showing normal spermatogenesis (Fig. 1), seminiferous tubules were present and smears displayed good cellularity. The differential count (Table-2) revealed upto 40.0% Sertoli cells. The spermatogenetic cells constituted more than 50.0% with spermatozoa being predominating one.

Sertoli cell only syndrome (Fig. 2) is the commonest cytological diagnosis and smears are slightly less cellular than that of normal spermatogenesis. Smears showed sheets and clusters of Sertoli cells with no or occasional spermatogenetic cells. Sertoli cells constituted 89.0–100.0%.

Ten cases of hypospermatogenesis (Fig. 3) showed cellular smear and constituted more than 56.0% Sertoli cells and less than 25% spermatozoa. Table-3 shows the differential count.

Table-3: Differential count in hypospermatogenesis

Cell types	Range (%)	Mean
Sertoli cells	56.0 – 74.0	63.7
Spermatogonia	0 – 2.0	0.8
Spermatocyte	2.0 – 6.0	5.1
Spermatids	5.0 – 13.0	9.2

In maturation arrest (Fig. 4), the spermatozoa were markedly reduced; Table-4 shows the differential count.

In tubular / peritubular sclerosis (Fig. 5), smears revealed very low cellularity and tubules showed hyalinized material with isolated crumpled Sertoli cells and few or no spermatozoa.

Various indices in different categories are mentioned in Table-5. The mean spermatic index in maturation arrest is significantly different from that in hypospermatogenesis (P value 0.001) and normal spermatogenesis (P value 0001). The mean Sertoli cell index is lowest in normal spermatogenesis and progressively increasing in maturation arrest, hypospermatogenesis, tubular / peritubular sclerosis and Sertoli cell only syndrome. The mean sperm – Sertoli index is highest in normal spermatogenesis and gradually decreasing in maturation arrest, hypospermatogenesis, tubular / peritubular sclerosis and Sertoli cell only syndrome.

Testicular biopsy was available only in 11 cases, in which FNA was also performed before biopsy from the same testis. The finding is shown in Table-6. There was complete agreement between the cytological smears from FNA and histological sections obtained by biopsy. In 1 case, this was reported as inadequate smear, the biopsy revealed tubular / peritubular sclerosis. Cytohistological correlation was shown in Table-7.

Few complications noted after FNA procedure. Severe pain was observed in 2 out of 101 patients and hematoma in 3 patients. Pain was managed by intramuscular Diclofenac sodium injection. Hematoma resolved spontaneously.

Table-2: Differential count in normal spermatogenesis

Cell types	Range (%)	Mean
Sertoli cells	24.0 – 40.0	32.8
Spermatogonia	1.0 – 3.0	1.1
Spermatocyte	4.0– 14.0	7.3
Spermatids	12.0 – 21.0	14.7
Spermatozoa	35.0 – 50.0	43.3

Table-4: Differential count in maturation arrest

Cell types	Range (%)	Mean
Sertoli cells	28.0 – 63.0	38.3
Spermatogonia	1.0– 2.0	1.1
Spermatocyte	6.0 – 11.0	8.0
Spermatids	22.0 – 44.0	33.5
Spermatozoa	8.0 – 24.0	19.0

Table-5: Cell indices in different categories

Category	Spermatic index (mean)	Sertoli cell index (mean)	Sperm-Sertoli index (mean)
Normal spermatogenesis	55.5 – 71.4 (64.7)	31.5 – 66.6 (49.7)	94.5 – 187.5 (136.6)
Sertoli cell only syndrome	0 – 100 (44.5)	0 – 9900; α (3225.3)	0 – 5.5 (2.8)
Hypospermatogenesis	54.2 – 74 (60.4)	127.2 – 284.6 (184.1)	22.9 – 46.5 (30.6)
Maturation arrest	21.6 – 37.5 (30.1)	38.8 – 170.2 (70.5)	12.7 – 82.7 (56.1)
Tubular / peritubular sclerosis	0 – 35.2 (17.5)	316.6 – 9900; α (1985.6)	0 – 7.2 (3.6)

DISCUSSION

Male factor is responsible for 40.0–50.0% of infertility cases. The incidence of azoospermia was estimated at 17 per 1000 men in the range of 20–59 years in Jordan[11] and such type of data in Nepalese population is not available. In this study, the age range of azoospermic male is 21–51 years.

Testicular biopsy is well established as one of modalities of investigation in male infertility.[12,13] In view of the good correlation between histology of the testis and FNAC, the latter is gaining more popularity.[14] Although the utility of testicular aspiration in male infertility was first described in 1928,[15] it was only in the late 1980s that detailed descriptions of cytologic morphology of various cells seen in FNA smears of the testis appeared in the literature.[5,16] Nowadays, FNA is not only popular in assessing the testicular function in azoospermic males, but also has several advantages over biopsy.[6,9] This study has attempted to describe cell counts, indices on FNA smears and correlate with histologic findings seen in testicular biopsy.

Various cell types in FNA smears were identified by their typical morphology. The short lived secondary spermatocytes and early spermatids were not distinguished separately in this study. Overall cellularity, differential count and indices helped to classify correctly most of the cases. Normal spermatogenesis in this study was characterized by 35.0–50.0% spermatozoa and up to 40.0% Sertoli cells, which is comparable (Sertoli cells up to 40.0% and 38.0–54.0% spermatozoa) to the finding of Batra *et al*.[10] Maturation arrest is characterized by marked reduction in spermatozoa (mean 19.0%) and rise in spermatids (mean 33.5%). Batra *et al*[10] found mean spermatozoa 15.8% and spermatids 25.4% in maturation arrest. Hypospermatogenesis was characterized by more than 56.0% Sertoli cells and reduced counts of spermatogenetic cells in this study, while Sertoli cells count is more than 50% in the study of Batra VV *et* al.[10] Both Sertoli cell only syndrome and tubular / peritubular sclerosis showed markedly elevated Sertoli cells. Sertoli cell only syndrome was characterized by more than 88% Sertoli cells. Smears of Sertoli cell only syndrome were cellular and showed sheets of Sertoli cells, while in tubular / peritubular sclerosis, smears were less cellular and displayed fragments of atrophic (hyalinized) tubules with isolated crumpled Sertoli cells.

Table-6: Histopathological diagnosis in 11 cases

Category	No. of cases (%)
Normal spermatogenesis (Fig. 6)	3 (27.3)
Sertoli cell only syndrome (Fig. 7)	5 (45.5)
Hypospermatogenesis	1 (9.0)
Tubular / peritubular sclerosis (Fig. 8)	2 (18.2)
Total	**11 (100.0)**

A few studies have tried to use cell indices in an attempt to obtain objective information.[10,16] This study shows that differential count and cell indices can help in correctly classifying all cases. Spermatic index, Sertoli cell index and sperm - Sertoli cell index can distinguish normal spermatogenesis and abnormal spermatogenesis (maturation arrest, hypospermatogenesis and Sertoli cells only syndrome). Each index may contribute some diagnostic information. My finding suggests that spermatic index may be useful in differentiating normal spermatogenesis (p value 0.001) and hypospermatogenesis (p value 0.001) from maturation arrest. Sperm – Sertoli cell index may be useful in differentiating normal spermatogenesis from hypospermatogenesis and maturation arrest. A combination of differential counts and indices may be useful for quantitating spermatogenesis.[10]

The heterogeneity of the spermatogenetic process within the testis as well as between two testes requires sampling of both testes and multiple punctures. Single aspirate may not be truly representative.[17] However, some studies have described sampling of only one testis.[6] This study used sampling of only one testis because of lack of compliance by the patient. In one case, another testis was sampled on the next day and cytological diagnosis was similar on both testes.

This study showed Sertoli cell only syndrome is commonest finding in azoospermic males; in contrast to the finding of Batra VV et al that showed normal spermatogenesis is commonest diagnosis.

Table-7: Correlation between cytological and histological diagnosis

Cytological diagnosis (No. of cases)	Histological diagnosis			
	Sertoli cell only syndrome	Normal spermatogenesis	Hypospermatogenesis	Tubular/peritubular sclerosis
Sertoli cell only syndrome (5)	5			
Normal spermatogenesis (3)		3		
Hypospermatogenesis (1)			1	
Tubular/peritubular sclerosis (1)				1
Inadequate (1)				1

This study showed complete agreement between FNA and biopsy. In a study of Rammou – Kinia R *et al*,[18] the sensitivity and specificity of FNAC in assessing testicular function were 100.0%. A study of Mahajan AD[6] showed a 97.0% agreement between the dianoses from FNA and biopsy, while discordant dianoses between cytology and histology were encountered in 6% of specimens in another study.[19]

The limitation of testicular FNA cytology includes an inability to assess the status of the tubular basement membrane. This information is not important in infertility if the status of spermatogenesis is clear.

In my opinion, FNA cytology in experienced hands may be preferable to biopsy, which can cause adhesions, fibrosis and may subsequently cause testicular sperm extraction difficult.

The technique of FNA cytology is a simple, quick, inexpensive and minimally invasive outpatient procedure. The sample obtained is more representative than a biopsy. FNA, in experienced hands, by calculating percentage cell counts and indices, provides reliable diagnosis in patients with azoospermia.

REFERENCE

1. Jarow JP, Espeland MA, Lipshultz LI. Evaluation of the azoospermic patient. *J Urol* 1989; 142: 62-5.
2. Hotchkiss RS. Fertility in man. Philadelphia: Lippincott. 1944: 206.
3. Obrant KO, Persson PS. Zytologis Che untersuchung des Hodens durch. Aspiration biopsie, Zur Beuteilung der. *Fetil Urol Int'l* 1965; 20: 176-89.
4. Persson PS, Ahren C, Obrant KO. Aspiration biopsy smear of testis in azoospermia. *Scand J Uorl Nephrol* 1971; 5: 22-6.
5. Schench U, Schill WB. Cytology of human seminiferous tubules. *Acta Cytol* 1998; 32: 689-96.
6. Mahajan AD, Ali NI, Walwalkar SJ, Rege JD, Pathak HR. The role of fine needle aspiration cytology of the testis in the diagnostic evaluation of infertility. *Brit J Urol Int'l* 1999; 84: 485-8.
7. Kumar R, Gautam G, Gupta NP *et al*. Role of testicular fine needle aspiration cytology in infertile men with clinically obstructive azoospermia. *Natl Med J India* 2006; 19:18-20.
8. Al-Jitawi SA, AI-Ramahi SA, Hakooz B. Diagnostic role of testicular fine needle aspiration biopsy in male infertility. *Acta Cytol* 1997; 41: 1705-8.
9. Papic Z, Katona G, Skrabalo Z. The cytologic identification and quantitation of testicular cell subtypes: Reproducibility and relation to histologic findings in the diagnosis of male infertility. *Acta Cytol* 1988; 32: 697-706.
10. Batra VV, Khadgawat R, Agrawal A *et al*. Correlation of cell counts and indices in testicular FNA with histology in male infertility. *Acta Cytol* 1999; 43: 617-23.
11. Dajani YF, Kilani Z. Role of testicular fine needle aspiration in the diagnosis of azoospermia. *Int'l J Androl* 1998; 21: 295-300.
12. Levis HS. Testicular biopsy in the study of male infertility: Its current usefulness, histologic techniques and prospects for future. *Hum Pathol* 1979; 10: 569-84.
13. Wong TW, Straus FH, Warner NE. Testicular biopsy in the study of male infertility:I. Testicular causes of infertility. *Arch Pathol* 1973: 95: 151-9.
14. Ali MA, Akhtar M, Woodhouse N, Burgess A, Faulker C, HuqM. Role of testicular fine needle aspiration biopsy in the evaluation of male infertility: Cytologic and histologic correlation. *Diagn Cytopathol* 1991; 7: 128-31.
15. Huhner M. Aspiration of the testicle in the diagnosis and prognosis of sterlity. *J Urol* 1928; 9: 31-41.
16. Han U, Adabað A, Köybaþioðlu F, Onal BU. Clinical value of cell counts and indices in testicular fine needle aspiration cytology in primary infertility. *Anal Quant Cytol Histol* 2006; 28: 331-6.
17. Skakkebaek NE, Hammen R, Philip H, RebbeH. Quantitation of human seminiferous epithelium. Histological studies in 44 infertile men and controls with normal chromosomal complements. *Acta Pathol Microbiol Scand* 1973; 81: 97-111.
18. Rammou – Kinia R, Anagnostopolou I, Tassiopoulos F, Lykourinas M. Fine needle aspiration of the testis, correlation between cytology and histology. *Acta Cytol* 1999; 43: 991-8.
19. Meng MV, Cha I, Ljung BM, Turek PJ. Testicular fine needle aspiration in infertile men: Correlation of cytologic pattern with biopsy histology. *Amer J Surg Pathol* 2001; 25: 71-9.