

Cytological findings of testicular fine needle aspiration in a sample of azoospermic Iraqi patients

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Abstract

Background: Azoospermia account for about 10–15% of male infertility cases. It denotes the final result of different testicular alterations, ranging from non-obstructive to obstructive azoospermia which could be corrected surgically. The former is associated with impairment of spermatogenic process, including hypospermatogenesis, maturation arrest and complete absence of germ cells-Sertoli cell-only syndrome (SCOS). To ascertain such azoospermia aetiology, Testicular biopsy is considered as the standard method. However, the popularity of Fine needle aspiration (FNA) cytology has gained as simple and minimally invasive way that can help in assessing testicular function accurately.

Aims: To determine causes of azoospermia, by assessing the cytological findings in testicular FNA.

Patients and Methods: A prospective study for 95 azoospermic patients was conducted from Jan 2010-Dec 2011 at private laboratory using FNA procedure. Here, detailed history and physical examination were done along with semen analysis to confirm true azoospermia. Both testes were aspirated to obviate sampling errors when appropriate. Routine Haematoxylin and Eosin staining was also performed on the smears.

Results: Adequate samples were obtained from 84 (88.4%) cases, while 11 patients (11.6%) had scanty smears where cytological diagnosis could not be obtained. These adequate smears were categorized as normal spermatogenesis in 38 (45.2%) patients, maturation arrest at spermatocyte/spermatid level, and Sertoli cell only syndrome in 27 (32.2%) and 19 (22.6%) patients, respectively.

Conclusions: FNAC could permit characterization of specific cytological pictures related to testicular damage nature. It is able to identify different azoospermic subjects and represents a reliable and prognostic parameter of seminiferous epithelium status.

Keywords: Azoospermia, Fine needle aspiration, Spermatocytic arrest, Germ cell aplasia

INTRODUCTION

Male factors are responsible for about half of all infertility cases. Azoospermia, being the first abnormal investigation, mandates detailed clinical evaluation and investigations accounting for about 10–15% of these cases.^[1, 2]

Azoospermia represents the final result of different testicular alterations, ranging from obstructive azoospermia in which there is normal spermatogenesis with seminal tract obstruction or absence of vas deferens, this type of azoospermia is one of the surgically correctable causes of male infertility and is associated with a good outcome, another type of azoospermia called (non obstructive azoospermia) in which there is impairment of the spermatogenic process, including hypospermatogenesis, maturation arrest and complete absence of germ cells-Sertoli cell-only syndrome(SCOS), those patients do not benefit from surgery.^[2, 3]

Until recently testicular biopsy was the standard method for ascertaining the aetiology of azoospermia. As an alternative, testicular fine needle aspiration (FNA) has gained increasing popularity as a simple, minimally invasive procedure that can help in assessing testicular function accurately, On the other hand, limited awareness of the usefulness of the technique, lack of expertise in aspiration and interpretation of the cytological variations as well as paucity of information about architectural details on cytology remain limiting factors for more widespread adoption of this modality.^[4]

Fine needle aspiration cytology (FNAC) of the testes was proposed in 1992 and has been demonstrated to represent a minimally invasive and reliable parameter in the study of the seminiferous epithelium and spermatogenic process in severely infertile men.^[5-7]

Testicular cytological analysis is relatively simple and allows the identification of different clinical groups of infertile subjects and different kinds of testicular tubular alteration in azoospermic subjects.^[4, 5]

The assessment of these patients encompasses a detailed history, physical examination and the performance of various investigations. The latter includes blood tests [chromosomal study analysis, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin and testosterone, ultrasound, vasography and usually open testicular biopsy].^[8]

This study was aimed at addressing the question whether testicular FNA may be used as a first-line diagnostic

modality in azoospermia & to determine the types of aspirate in patients with azoospermia.

PATIENTS AND METHODS

Patient selection

Fine needle aspiration procedure was performed in 95 azoospermic patients from January 2010 through December 2011, a detailed history and physical examination was performed on all patients. In addition, semen analysis was also performed to confirm true azoospermia. Hormonal evaluation including testosterone and FSH levels were obtained in the majority of patients.

FNAC technique

The FNAC procedure was performed as previously described (Turek et al. 1997).^[9] Briefly, the scrotal skin was cleaned & spermatic cord infiltrated with local anesthesia, the testis was positioned with the epididymis directed posteriorly. The scrotal skin was stretched taut over the testis by wrapping the scrotal skin behind the testis with a sponge.

The planned aspiration sites were marked on the scrotal skin overlying the testis.

FNA was done with 23 gauge fine needle & 10ml syringe & using the established suction- cutting technique (in & out movements varying from 5-8mm were used to aspirate the tissue), four to five needle excursions were made at each site of aspiration, Then suction was released, & the tissue was expelled onto a slide, gently smeared, & fixed immediately in 95% ethyl alcohol.

Pressure was applied to each site after aspiration for hemostasis. To obviate sampling errors both testes were aspirated, except when contraindicated. Contraindications for bilateral testicular sampling include the presence of local skin infection, hydrocele, orchialgia or a previous biopsy.

Routine Hematoxylin & Eosin stain was performed on the smears.

FNA interpretation

Each stained FNA cytological smear was interpreted for:

- (i) The presence or absence of mature spermatozoa with tails
- (ii) Specimen adequacy, as previously reported, an adequate, and informative, FNA specimen was defined as one that contained at least 100 clusters of 20 or more cells or at least 2000 well-dispersed testicular cells.^[9]

RESULT

The FNA was performed in 95 cases of azoospermia (38 of cases in 2010 & the rest (47 of cases) on 2011) (Table 1).

Table 1. Distribution of cases in present study according to years.

Year	Number of cases
2010	38
2011	47

Their age ranged from 16 to 35 years, with period of infertility more than one year.

Adequate sample was obtained in 84 (88.4%) cases; other eleven (11.6%) cases revealed scant smears where cytological diagnosis could not be made. (Table 2)

Table 2. Percent of adequacy of testicular smears

Type of samples	No. of smears	% of smears
Adequate smear	84	88.4%
Scant smear	11	11.6%

Adequate smears were categorized on cytological examination into: (Table 3).

1. Normal spermatogenesis in 38 (45.2%) patients.
2. Maturation arrest at the spermatocyte / spermatid level in 27(32.2%).
3. Sertoli cell only syndrome in 17(22.6%) patients.

Normal spermatogenesis of testes on FNA revealed all germ cell maturation steps from spermatogonia till mature spermatozoa. (Figure 1).^[10]

Table 3. Categories of testicular FNA in azoospermia.

Morphological diagnosis	No. of cases	% of cases
Normal spermatogenesis	38	45.2%
Maturation arrest	29	34.5%
Sertoli cell only	17	20.3%

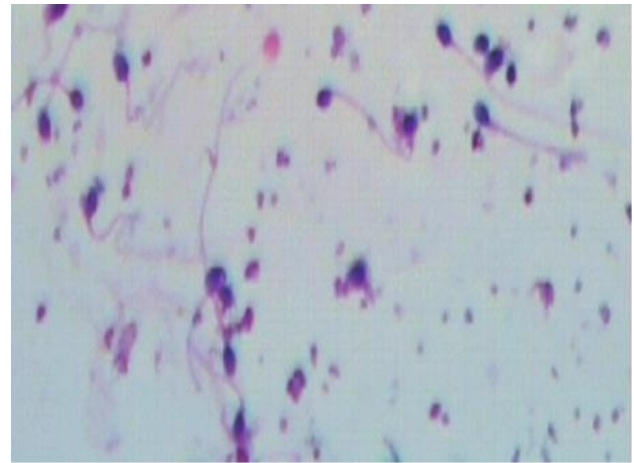


Figure 1. Showing spermatids & mature spermatozoa (H&E, X400).

Maturation arrest category shows no spermatozoa, with presence of immature germ cells, including several primary spermatocytes & spermatids (Figure 2).^[11]

Sertoli cell only syndrome (germ cell aplasia) on FNA of testes showing only sertoli cells. (Figure 3).^[11]

Spermatogonia were seen as large cells with round nuclei and finely granular chromatin with a thin rim of cytoplasm.^[10]

Primary spermatocytes had coarse nuclear chromatin with scant to moderate cytoplasm and well-defined cell boundaries.

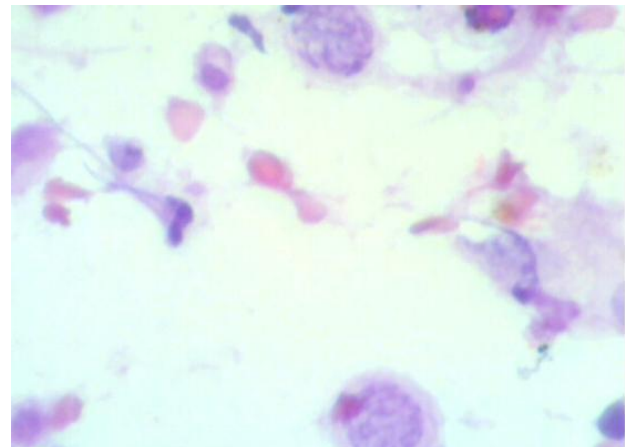


Figure 2. Showing spermatocytes (H&E, oil immersion X1000).

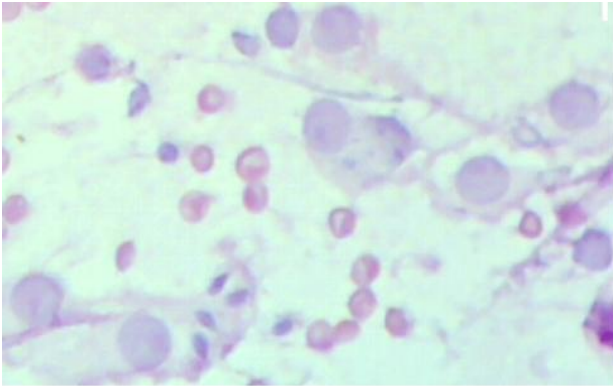


Figure 3. Showing sertoli cells (H&E, X400).

Spermatids showed uniform granular nuclear chromatin. The cytoplasm was more abundant in immature forms, gradually diminishing as cells matured (Figure 1).

Sertoli cells showed fine vacuolated ill-defined cytoplasm and indistinct cell borders with round nuclei (Figure 3).^[10]

DISCUSSION

Azoospermia is present in about 10–15% of men evaluated for infertility and represents the final result of different testicular alterations, ranging from normal spermatogenesis with seminal tract obstruction or absence of vas deferens (obstructive azoospermia) to different problems of the spermatogenic process including hypospermatogenesis, maturation arrest and complete absence of germ cells or SCOs (nonobstructive azoospermia).^[1, 11-13]

Testicular FNA is a simple, minimally invasive alternative procedure that can diagnose accurately testicular histology. In addition to other adjuncts like testicular volume and the hormonal status, it may help in differentiating obstructive from non-obstructive azoospermia. In cases of non-obstructive azoospermia, multiple passes with a fine needle may be performed instead of open testicular biopsy for sperm extraction for artificial reproductive techniques.^[4]

Testicular FNA has not become widely accepted as a routine, this may be because it is considered unreliable, or likely to cause trauma and haematomas, but a further relevant factor is probably the lack of cytologists with expertise in this field and the paucity of written information. However, the cytology of seminiferous epithelium has been recently described (Papic et al., 1988; Schenk and Schill, 1993),^[14, 15] and in each case on smears taken from open biopsies. Gottschalk-Sabag et al. (1993) described the cytology of testicular FNA and

compared their findings to the histology of concurrent open biopsies. In their series of 54 cases they defined three diagnostic categories: normal, spermatogenic arrest, and Sertoli cell only.^[16]

In our study, FNA was done for 95 cases of azoospermia (diagnosed on semen analysis) & adequate aspirates are grouped into three diagnostic types including normal spermatogenesis in 38 patients (45.2%), maturation arrest at the spermatocyte / spermatid level in 27(32.2%), and sertoli cell only syndrome in 19(22.6%) patients.

Normal spermatogenesis pattern represents the highest percent of cytological pattern of FNAC of testes in the present study & these results are supported by many other studies.^[4, 10, 12, 17-20]

the highest percent of normal spermatogenesis among other cytological patterns of testicular FNA is probably due to including of mild & moderate hypospermatogenesis cases within this group, which cannot be detected easily on FNAC of testes & also probably due to the presence of oligospermia cases within this group that are diagnosed incorrectly as azoospermia on semen analysis.^[4, 8, 17, 21]

Spermatocytic arrest & Sertoli cell only syndrome patterns in testicular FNA of azoospermic patient are the major two pathological findings (54.8%) in the present study; these findings are confirmed by the results of many previous studies.^[4, 8, 17, 18, 19, 21, 22]

These results reflect the heterogeneity in FNA readings between different areas of the testis, differences in staining characteristics of testicular FNA.

Conclusion

FNAC permits the characterization of specific cytological pictures that are related to the nature of testicular damage, such as Sertoli cells only syndrome, hypospermatogenesis and maturation arrest. Therefore, cytological evaluation is able to identify different groups of azoospermic subjects and represents a reliable and prognostic parameter of the status of the seminiferous epithelium.

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