CYTOMORPHOLOGICAL SPECTRUM OF TESTICULAR FINE NEEDLE ASPIRATION CYTOLOGY IN CASES OF AZOOSPERMIA

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ABSTRACT

Objective: To determine the frequency of morphological spectrum of FNAC in cases of azoospermia.

Study Design: Descriptive, cross sectional study.

Setting: Department of Pathology, Combined Military Hospital, Multan, from 15th December 2016 to 15th June 2017.

Materials & Methods: A total of 112 patients were selected according to inclusion and exclusion criteria, that is age range between 20-50 years with history of azoospermia for at least 1 month on the basis of semen analysis. Patients with already known diagnosis, patients with history of orchiectomy or patients with history of radiotherapy and chemotherapy were excluded. Patients were informed about the procedure, their consent for the procedure and for participating in our study were taken. Procedure was performed under spermatic cord block, slides were prepared and reported by consultant histopathologist.

Results: Mean age was 31.6 years with standard deviation of ±5.91. All of the patients were married. Only 10 out of 112 patients had history of diabetes mellitus and only 5 out of 112 patients had family history of azoospermia. Mean BMI was 22.74 with standard deviation of ±2.95. The mean duration of marriage was 4.62 years with standard deviation of ±3.09 and mean duration of azoospermia was 2.69 years with standard deviation of ±2.23. Among the patients, 109 out of 112 patients had diagnostic FNAC finding while 3 out of 109 patients yielded unsatisfactory results. Among the cytomorphological patterns, hypospermatogenesis was the most common pattern which was observed in 32.1% of the patients. Normal spermatogenesis was seen in 18.8 percent of patients, maturation arrest was seen in 19.6% patients, sertoli cells only was seen in 26.8% patients. 2.7% patients had unsatisfactory sample. A non-significant statistical association was seen among age, disease duration, diabetes and family history with p value > 0.05.

Conclusion: This study was conducted to ascertain cytomorphological patterns of testicular tissue among targeted population and to determine the frequencies of these patterns in our population. This study concluded that FNAC testis is a satisfactory procedure for evaluating the cause of azoospermia and FNAC sample in 97.3% of the patient's was satisfactory for evaluation. Hypospermatogenesis was the most common cytomorphological pattern seen in 32.1% of the patients which was followed by sertoli cells examine only which was seen in 26.8% of the patients. Non significant association was found between patient's age, duration of disease, family history or diabetes mellitus with any of the cytomorphological pattern.

Keywords: Azoospermia, Cytomorphology, FNAC.

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INTRODUCTION

Infertility is defined as failure to conceive after 1 year of coital activity with same sexual partner without contraception [1,2]. Infertility is secondary to male, female or both causes [3]. Male infertility usually presents as azoospermia clinically which is seen among 1% of all men and 15-20% of men who seek medical attention [4]. Azoospermia is categorized into pretesticular, testicular and post-testicular types [5,6]. Among these categories, testicular azoospermia is usually irreversible while pretesticular causes (mostly due to hormonal imbalance) and post-testicular causes (usually obstructive) show good response to hormone and surgical treatment respectively [7]. Varicocele is the only exception which is testicular cause of azoospermia and shows good response to treatment [6]. This categorization of causes of azoospermia is helpful clinically, because previously all individuals who used to present with azoospermia were put on

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hormonal treatment and if no clinical and laboratory response was seen, they were labeled as sterile and sperm donation used to be the only option for them, however due to categorization of these causes, we can now confidently choose the patients for whom treatments are available [8].

The evaluation of an infertile male starts with clinical history, physical examination, semen analysis which includes sperm count, sperm motility and leukocyte quantitation and in some cases presence of antisperm antibodies [5,8]. Tests of sperm function include evaluation of cervical mucus interaction, ova penetration and the hemizonal assay. Additional tests are performed in selective cases which are transrectal ultrasonography, venography and testicular biopsy [9,10].

Testicular biopsy is the usual recommendation for differentiation between testicular and non-testicular causes of azoospermia and open testicular biopsy is preferred over punch biopsy because of its better diagnostic yield [6,10]. However, in recent years, fine needle aspiration cytology (FNAC) has been established as an important modality in the diagnosis and management of superficial and deep-seated lesions of different anatomical locations [11].

It has also been proposed as an alternative technique to surgical biopsy in cases of azoospermia in recent studies. In a study conducted by Qublan HS [12], a correlation system between biopsy and testicular FNAC was proposed in which 4 morphological patterns on cytological examination were identified which were interpreted as normal spermatogenesis, hypospermatogenesis, maturation arrest and sertoli cells only/germ cell aplasia which were present in 20.6%, 26.5%, 23.5% and 29.4% respectively. These patterns are based on three indices which are spermatic index, sertoli cell index and sperm-sertoli cell index. In a recent review article by Alam MA [5], a complete procedure and morphological spectrum has been proposed. FNAC has been suggested as a reliable technique [13], and in an article by Srivastava A, FNAC has been proposed as the only necessary study for evaluation of patient who presents with azoospermia [14].

FNAC has certain advantages over biopsy. It is a simple, quick and inexpensive outpatient procedure and requires special setting and anesthesia [15]. Its diagnostic accuracy in detecting spermatogenesis is better than biopsy because for FNAC, multiple sites of testis can be sampled while biopsy is taken from only one site [16]. Its greatest value is in the evaluation of non-obstructive azoospermic males where it can conserve tissue of an already failing organ [17].

Previously scanty data is available on this aspect of FNAC and very few international studies are available on this subject. This study will help in determining the frequencies of cytomorphological patterns in our population which is important because certain diseases such as tuberculosis [18] and mumps [19] are more prevalent than rest of the world, which are non-testicular obstructive causes of azoospermia. This study will also help in increasing the awareness among clinicians and will be helpful for patients because of it being more cost effective and safer diagnostic technique.

MATERIALS AND METHODS

This descriptive cross-sectional study was carried out at Department of Pathology, Combined Military Hospital, Multan from 15th December 2016 to 15th June 2017, after the approval of ethical committee. A total of 112 patients were selected after taking informed consent, between 20 to 50 years of age with history of azoospermia for at least 1 month on semen analysis. Sample size was calculated using WHO sample size calculator. The patients were

selected by non-probability, consecutive sampling technique. Their demographic (name and age) and clinical data (history, clinical examination and semen analysis) was noted on a predesigned performa. Patients with already known diagnosis, history of orchiectomy and patients on radiotherapy or chemotherapy were not included in the study. They were informed about the procedure which was to be performed under local anesthesia. Scrotal skin was cleaned and spermatic cord block was achieved by 5-7 ml of 2% Lidocaine solution. Once anesthesia was achieved, testis was positioned with epididymis and vas deferens directed posteriorly, scrotal skin was stretched over the testis. Testis was aspirated at three different sites; upper, middle and lower part, using 21-23 guage needle with 10-20 ml syringe attached to it. Both testes were aspirated and material was spread over slides, marked for both sides. Slides were stained, using Hematoxilin and Eosin Stain as well as Diff Quick stain and were examined by Consultant Histopathologist for reporting, who had an experience of at least 5 years in this field.

Data was analyzed using computer software SPSS version 22.0. The quantitative variables i.e. age and duration of azoospermia were presented by calculating mean and standard deviation. The qualitative variable i.e. history of diabetes mellitus, family history, hypospermatogenesis, germ cell aplasia, maturation arrest and normal spermatogenesis were presented by calculating frequency and percentages. Further effect modifiers like age, duration of disease, history of diabetes mellitus and family history were controlled through stratification. Post stratification, chi square test was applied. P value less than or equal to 0.05 was considered at level of significance.

RESULTS

In this study, age range was from 22-50 years with mean age of 31.65 and standard deviation of ±5.9. The patients were divided into three groups on the basis of age. Group 1 included patients ranging from 21-30 years, Group 2 included patients range from age 31-40 years and Group 3 included patients ranging from 41-46 years. Our study results indicated that majority of our patients' i.e. 56 (50%) belonged to group 1. All the patients in our study were married. Duration of azoospermia ranged from 1 year to 15 years with mean duration of 2.7 years and standard deviation of ±2.2 years.

Only 10 out of 112 patients (8.9%) had a history of diabetes mellitus and only 5 out of 112 patients (4.5%) had positive family history of azoospermia among blood relatives. FNAC testis was diagnostically adequate in 109 out of 112 (97.3%) patients. In 3 out of 112 patients (2.7%), FNAC testis was not diagnostically adequate to give any cytomorphological pattern.

Among cytomorphological patterns (Figure-1 a,b,c,d), hypospermatogenesis had the highest frequency (n=36) and was seen in 32.1% of the patients. Normal spermatogenesis (n=21) was seen in 18.8% of the patients. Maturation arrest (n=22) was seen in 19.6% of the patients and sertoli cells only (n=30) was seen in 26.8% of the patients. 2.7% (n=3) samples were inadequate for opinion. These cytomorphological patterns have been stratified with regards to age, duration of azoospermia, diabetes and family history (Table-1). A non-significant statistical association was seen among age, disease duration, diabetes and family history with *P* value > 0.05.

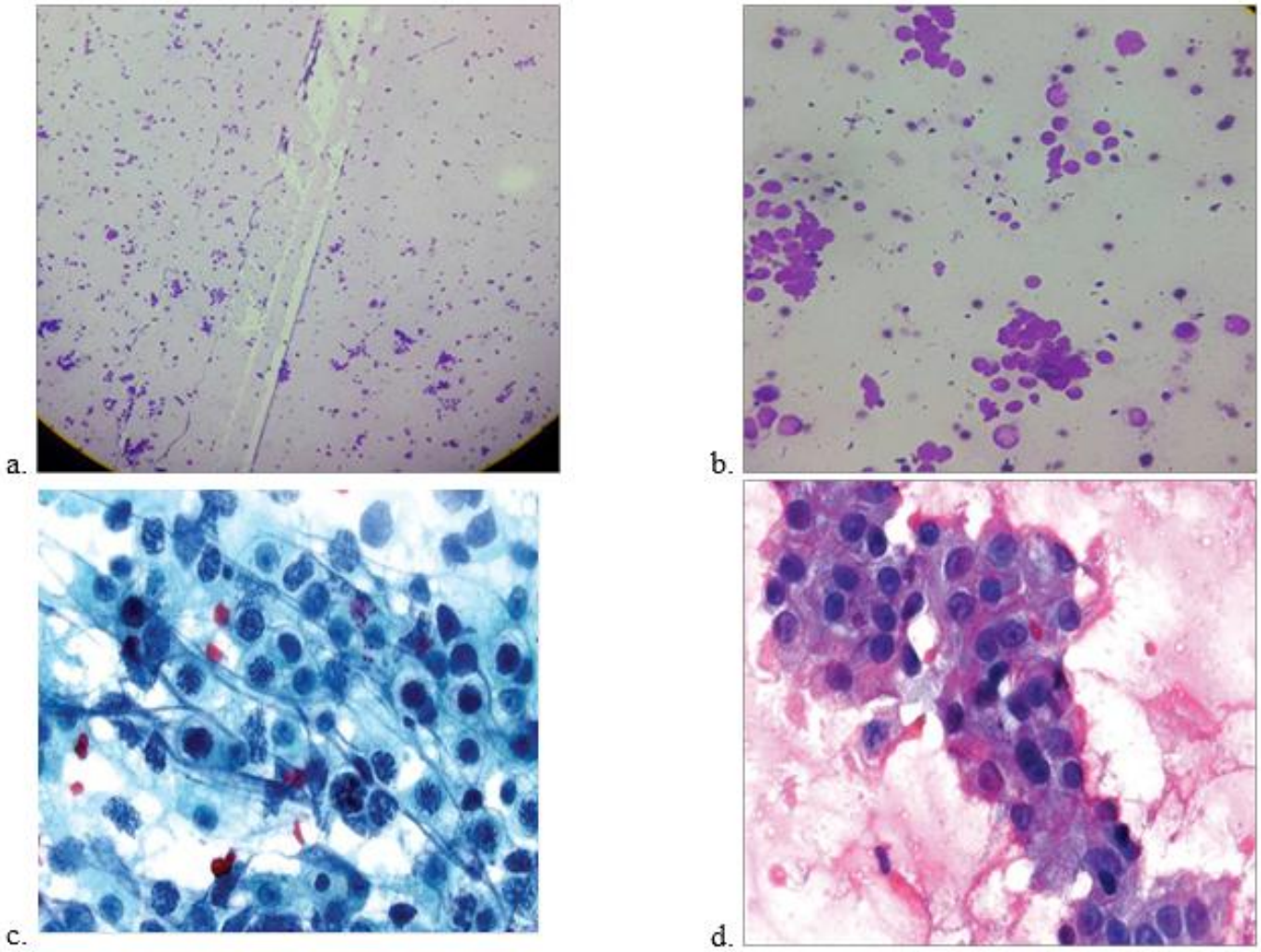

Figure 1. Photomicrographs of Cytomorphological Patterns

a. Normal Spermatogenesis, b. Hypospermatogenesis, c. Maturation Arrest, d. Sertoli Cells only

Table-1: Distribution of cases according to effect modifiers (Mean Age 31.65 ± 5.9 years, n=112)

Effect modifiers		Cases (n=112)	Percentage (%)	Cytomorphological Pattern			
				Normal Spermatogenesis	Hyposperm-atogenesis	Maturation Arrest	Sertoli Cells Only
Age groups (Yrs)	21-30	56	50	12	20	9	15
	31-40	45	40.2	8	12	10	12
	41-50	11	9.8	1	4	3	3
Duration of Azoospermia	<5	96	85.7	19	30	20	25
	>5	16	14.3	2	6	2	5
History of Diabetes Mellitus		10	8.9	2	1	3	4
Family History		5	4.5	1	3	0	1

*Chi-square test applied, **3 cases were non-diagnostic

DISCUSSION

Azoospermia results in infertility, which is cause of significant social and psychological problem for the patient suffering from it. A study published in 2013, Journal of Pakistan Medical Association [20] regarding the epidemiology of Infertility. 500 male partners of infertile couples were selected for testing. 104 (20.8%) had to be left out either because of their unwillingness or inability to pass semen. The study sample comprised of 396 (response rate of 79.2%). Among these participants, normospermia was observed in 293 (73.99%) males, azoospermia in 59 (14.89%) and oligospermia in 44 (11.11%) of the participants. This study was consistent with another study [21] (n=1521) conducted in Pakistan (publication) where azoospermia was found among 13.3% of the male partners. No specific relation to age was found, and this problem is evenly present among all patients with all ages of reproductive life. It turns out that prevalence of azoospermia in Pakistan is not very different from other parts of the world, where the prevalence among infertile couples is between 10-15% [6].

Cytomorphological patterns which are detected in patients suffering from azoospermia include normal spermatogenesis, hypospermatogenesis, maturation arrest and sertoli cells only. Atrophic pattern which can be diagnosed on histopathology of testicular biopsy cannot be confidently diagnosed on FNAC because it does not fulfill the criteria of adequacy [5,12]. FNAC testis is an indication for patient with Azoospermia to differentiate between obstructive, hormonal and primary testicular causes of azoospermia [6]. This procedure has high sensitivity and specificity, lower cost, and lower incidence chance of complications [15]. FNAC testis also has the advantage to detect site of most advanced spermatogenesis, while testicular biopsy can be reserved for sperm retrieval techniques later on [22].

Various international and region studies have been published on this topic, however no such study about the frequency of these cytomorphological patterns have been published in Pakistan. Our study included a total of 112 patients suffering from azoospermia. Mean age of these patients was 31.65 years with standard deviation of ±5.9 with minimum age of 22 years and maximum age of 46 years. A study conducted by Ahmad MSU et al [13] in Bangladesh reported the mean age of 32.7 with age range between 24 to 50 years. These results are close to our study results. Another study by Bettella A et al [23], conducted in Italy, the mean age of the patients was 37.6 ± 3.3 years. Their mean age was higher, probably because of the culture of relatively late marriage in Western countries which results in later appearance of problem. Another such study conducted in Iraq by Ali SA et al[24], reported the mean age of 42.5 years which is higher than our study, because they also included 6 patients over the age of 50 and 3 patients over the age of 60 which resulted in higher mean age.

All of the patients who visited to our laboratory for evaluation were married because of infertility problem, most of the times is encountered only after marriage. Duration of marriage ranged between 1 year and 17 years. Mean duration of marriage was 4.6 years with standard deviation of ± 3.1 years. Duration of Azoospermia ranged between 1 year to 15 years, with mean duration of azoospermia of 2.7 years and standard deviation of ±2.2 years. Single such study conducted in Iraq by Al-Dabbagh AA et al [16], where mean duration of disease was reported to be 6 years, which is higher than our study but is similar to our study when we consider the fact that mean duration of marriage in our study is 5 years and that men in our culture start

their investigations only after all investigations in their female partner are clear.

Among cytomorphological patterns, hypospermatogenesis had the highest frequency (n=36 and was seen in 32.1% of the patients. Maturation arrest was seen in 19.6% (n=22) of the patients and sertoli cells only was seen in 26.8 % of the patients. Among different studies on this topic, different cytomorphological patterns have been used, most common among them are the four patterns used in our study, however in some of the studies, distinction between normal spermatogenesis and hypospermatogenesis was not made, while in other studies, the diagnosis of atrophic testis was used instead of inadequate for opinion despite the lack of cellularity and failure to fulfill adequacy criteria.

The inclusion criteria in our study was as general as in the studies by Qublan HS [12] and Ali SA [24], the results are almost similar with the exception the sertoli cells only was the most predominant pattern among those two studies, while in our study, it was the second most predominant pattern, with hypospermatogenesis having slightly higher frequency than sertoli cells only. The difference however is not very significant, because sertoli cells only and hypospermatogenesis are the two predominant patterns with almost similar frequencies in all these three studies.

CONCLUSION

This study concluded that majority of patients with azoospermia have the hope of becoming fathers, and they should be investigated further for the status of spermatogenesis. More than half of the patients (50.9%) did have mature sperms in their testis and about 20% of patients had the ability to produce sperms. Therefore, it is not a right practice to declare a patient of azoospermia to be sterile after two or three semen analyses. Hypospermatogenesis is the most prevalent cytomorphological pattern among the patients who present with azoospermia, followed by sertoli cells only in our population. FNAC testis is a reliable diagnostic procedure for patients with azoospermia to detect spermatogenesis as well as cytomorphological patterns when established guidelines are followed. It gives diagnostic yield in majority of patients (97.3% in our study). Furthermore, no significant association has been found between any cytomorphological pattern and conditions such as age, duration of azoospermia, family history, diabetes mellitus and obesity.

AUTHORS CONTRIBUTION

Muhammad Umar Ali Khan: Entire research work, sample collection and writeup

Muhammad Tahir Khadim: Concept and overall supervision

Syed Salman Ali: Analysis, literature review and writeup

Muhammad Zubair: Literature review

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