

Sperm Collection and Tissue Diagnosis Accuracy of FNAC Vs Open Testicular Biopsy

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ABSTRACT

Aim: To make diagnostic comparison between testicular open biopsy and fine-needle aspiration (FNA) in male patients who were diagnosed with medical condition like infertility and having azoospermia or severe oligozoospermia. More over this case study also made critical analysis of sperm extraction by testicles through technique FNA.

Place and duration of study: Muhammad Islam teaching hospital Gujranwala, October 2019 to February 2020

Study design: Comparative Study

Method: In this case research, about 110 testes of 60 patients were included in this case research. 48 patients were diagnosed with azoospermia out of 60, then 11 were diagnosed with oligozoospermia and 5 with oligozoospermia who were underwent orchidectomy procedure because of presence of prostate cancer were listed in this research. Two semen analysis tests were taken from each patient for detailed series of laboratory and clinical tests. For the test, FNA, a needle was selected that was 18mm, 24 gauge and 20-ml for smear extraction and smear was stained with Giemsa & Papanicolaou .

Result: After FNA procedure, an open biopsy was done in all patients and the obtained samples were dyed with haematoxylineos in stain. Under light microscope, both the smears and histological stains were keenly observed by same pathologist and comparison was made between two. The aspirate was not satisfactory in six testes where as in four testes there was spermatocytic arrest identified cytologically as compared to biopsies that showed testiffuse fibrosis. From 30 of 34 patients about (88.23%) spearmatozo can be extracted from patients having normal and hypospermatogenesis, where as in case of 38 patients, spermatozo cannot be acquired wither by FNA or open biopsy.

Conclusion: Testicular FNA is a very useful diagnostic tool in infertile males as it is least invasive as compared to open biopsy technique

Keywords: Biopsy, Fine-needle aspiration, Male infertility

INTRODUCTION

Following factors should be kept in mind while evaluating male infertility like secondary sexual characteristics development, clinical examination of gonadal growth, hormonal investigations, semen analysis, and finally testing of testicular biopsy morphologically⁴. From all of the above factors, the most important one among them is the histopathology of testicular tissue¹² Testicular biopsies are mainly done in males having axoospermia but normal testicular size as well as follicle-stimulating hormone (FSH) levels in order to differentiate the fact that whether the azoospermia can be attributed to blockage in the efferent duct system or not. Charney was the first man who gave this concept but its clinical implementation was one by Hotchkiss⁸. From their ideas and clinically successful cases, now a days less invasive methods are being in practice for extracting testicular material like 'tricut' prostatic needle biopsy⁵ and secondly, fine-needle aspiration cytology⁹. The pioneers of FNA of testes were Obrant and Persson who introduce the world this least

invasive procedure by trying on 42 patients with clinically diagnosed azoospermia^{4, 11}. FNA is a simple, rapid and least invasive method along with no complications where as compared to FNA, testicular biopsy is a lengthy procedure with more invasive and painful for patient and requires operating accessories

This case study aimed at FNA experience VS. open biopsy technique in infertile males.

PATIENTS AND METHODS

There was selection of thirty eight infertile male having age ranged from 23 to 50 years with medical condition like azoospermia or severe oligozoospermia and sperm count <2 million/ml on two different male events and two men age ranged 68 and 70 years with prostate cancer at advanced stage along with bilateral orchidectomy were chosen as candidates in this research. The serum FSH level and physical examination were observed for all patients

About 110 testes underwent examination by biopsy and FNA technique under local anesthesia. All testes underwent operation and were pricked with 26-gauge needle at least three times. Moreover, the needle was

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pricked from distinct directions to be sure and on safer side that different samples were collected from each testes .Different directions were chosen for pricking needle with the aid of 20ml syringe. From the site that was opposite to the epididymis, the puncture was made in testes right in the middle. The six sides were prepared from the extracted smear. Three slides were chosen for air-drying that afterwards were all stained using May–Grunwald–Giemsa (MGG), whereas the remaining three slides were all fixed by aid of 95% ethyl alcohol and then staining of all these slides were done with the help of Papanicolaou’s method (Pap). A small specimen about 5mm in diameter was taken as sample from testes after puncture with the help of sharp scissors and after that sample was fixed in Bouin’s solution. Paraffin was used for embedding then sample was cut into sections of thickness 5–6- μ m which afterward were stained with the help of haematoxylen and Eosin stain. Separate examination was carried out for both the cytological and histological smears and then comparison was made with the help of light microscope by same pathologist at $\times 100$ and $\times 400$ magnification. Depending upon the dominating cells we categorize the histological diagnoses into four categories like

Stage 1: Normal spermatogenesis: Spermatogenesis was seen of all stages.

Stage 2: Hypospermatogenesis: decreased numbers in all stages were identified. Moreover in tubules some spermatozoa were also seen

Stage 3: Spermatogenic arrest: interruption of sperm maturation was seen in this state. Detection of Neither spermatozoa nor spermatids was seen. At the primary spermatocytes stage, the arrest was usually detected.

Stage 4: Sertoli-cell-only syndrome: this syndrome is detected by absence of germ cell and by presence of Sertoli and Leydig cells only.

All the candidates’ under operation were discharged on same day. The results obtained by research were evaluated statistically by using Kramer V and McNemar correlation tests.

RESULTS

It was concluded that from 44 infertile candidates under study and evaluation about 34 patients had azoospermia whereas rest four were diagnosed with oligozoospermia. When history of case study was taken it was found that from 60 patients about three were diagnosed with mumps orchitis, about four patients had orchidectomy unilateral due to atrophy in testes and detection of seminoma , whereas there was ligation of spermatic vein in four patients done due to varicocele, five patients were diagnosed with secondary infertility and two with prostate cancer . When physical examination was carried out, left varicocele was detected in eight patients with azoospermia whereas epididymal cysts were evident in two.

From histological evaluation, three cells were seen in human testis known as Sertoli cells, spermatogenic cells, and finally Leydig cells. In 12 of 254 smears obtained by fine needle aspiration there is incidence of seminiferous tubular fragments. Since it is difficult to aspire and extract interstitial tissue, Leydig cells were just found only in two candidates. Staining of smears was done with MGG or Pap method. Both these staining methods are same and some researcher’s think that these two staining methods

should be applied al together so that we can benefit from each method during evaluation and examination with the help of microscope.

From 99 of 110 testes, here was seen similarity between the smears cytologically between open biopsy and FNA technique (Fig. 1A,B). This was proved by Kramer V correlation test and V value came out to be 0.932. In four testes, it was impossible to do cytological evaluation due to the fact that aspiration was not sufficient and cells aspirated were lower in number. According to histology, assessment there was incidence of SCO syndrome in two candidates and incidence of normal spermatogenesis seen in the other two candidates. There was evidence of spermatogenic arrest in other three testes from cytological point of view, whereas from histological point of view, diffuse fibrosis was identified.

Fig.1: Normal spermatogenesis in histological sections (H&E100).

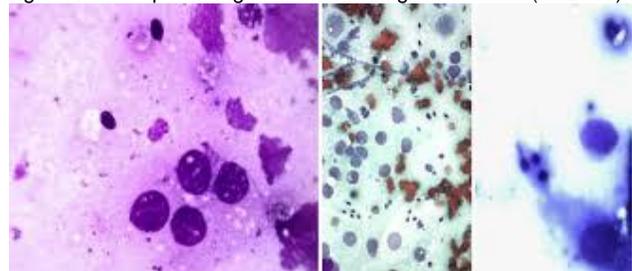


Fig. 2: Presentation of normal spermatogenesis in which spermatozoa was labeled with small arrows and Sertolicell by big arrow in fine-needle aspiration of testis

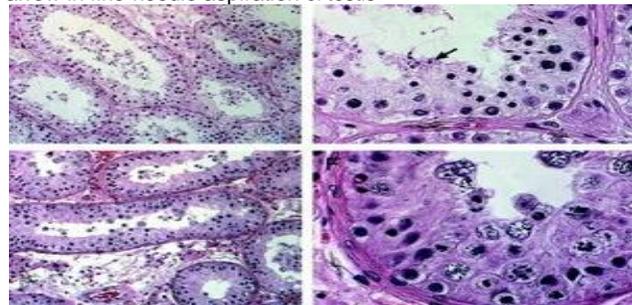


Fig 3: Sertolicell only in fine-needle aspiration

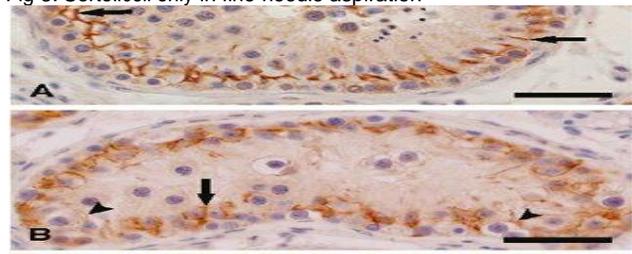


Table 1: Comparison of differential count between FNAC cytology and BIOPSY histology

Parameter	FNAC cytology (Mean %)	Biopsy histology (Mean %)
Sertoli Cell	82.4%	79%
Spermatogonia	78.7%	84%
Spermatocyte	85.2%	72%
Spermatids	88.1%	77%
Spermatozoa	90.5%	69%

With the help of testicular biopsy from 31 testes having normal or low sperm count, spermatozoa were identified whereas from FNA, there was presence of spermatozoa in 47 of 50 testes that was about 94%. There was absence of spermatozoa in one testes due to insufficient aspiration. In 43 testes of candidates having spermatogenic arrest and SCO syndrome, there was absence of spermatozoa as identified by open biopsy and FNA. Statistically, there was no difference in obtaining spermatozoa with either open biopsy or FNA (P 1).

There was presence of scrotal pain in forty candidates which afterwards was better which after 2 days. The presence of infected wound or haematoma was observed in none of the patients.

DISCUSSION

For evaluation of male infertility, method of choice these days is testicular biopsy specifically in patients suffering from oligozoospermia as well as azoospermia¹². In past testicular biopsy was done to diagnose azoospermia (unexplained) in patients but having normal serum hormonal level of FSH as well as physically normal sized testes in order to detect the defect in spermatogenesis whether due to testes impaired function or due to blockage in efferent duct system³. In ICSI single spermatozoon is enough. So, a single spermatozoa can be extraction by testicular biopsy thus making it possible to accommodate candidates having elevated serum hormonal level of FSH and physically smaller sized⁸. The local areas of spermatogenesis are clearly seen in men having raised FSH and smaller testicular size values. These local areas are also seen in men previously diagnosed as devoid of spermatogenesis e.g., having SCO syndrome². Moreover, it was proposed by researchers that in infertile men having azoospermia idiopathic, opting for ICSI treatment should be checked and examined for testicular carcinoma histologically especially in the areas where as the malignancy in testes tends to be at higher side⁴.

Alternative ways of obtaining and extracting spermatozoa other than open biopsy are testicular azoospermia and FNA technique. With the help of FNA good quality of spermatozoa from histologically and cytologically aspects can be obtained, although from clinical routine aspect it has not become a method of choice^{5,7}. FNA has superiority on open biopsy method in many ways like by doing various punctures from different sections of testes, more accurate and proper samples can be attained, sections obtained from FNA has less artifacts from histological point of view^{10,3}. And finally FNA is least invasive, cost friendly and a quick method as compared to open biopsy technique. However, still FNA is not method of choice in routine clinical setups because of the fear of local tumor implantation and needle trauma. It has been cleared with our research that FNA technique, there was no complications reported like trauma or such as haemorrhage from the FNA procedure. It is thought that cytological smear samples obtained by FNA do not give information regarding the interstitial tissue condition diameter of the tubule and basement membrane condition of the seminiferous tubules. Moreover, there is also less experienced cytologist in field of cytology that make it more difficult to extract all information. Although, from the paper published by Obrant & Persson (1965), the results mentioned were quite

satisfactory but still there was lack of detailed classification regarding different types of cell was noted. Schenck & Schill (1988) did research and presented cells classification of seminiferous epithelium (normal) that were visible in cytological material and then these results were compared with the histological workout. Gottschalk-Sabag et al. (1993) finally, published a paper in which he described detailed cytological histological as well as clinical correlation in details.

In our current research, the 52 cases showed good correlation, spermatogenesis and Sertoli cells were seen in 16 cases where as Leydig were seen in smears of four patients. This shows similarity in results with work of other researchers like¹¹. FNA smears of azoospermic men that showed normal spermatogenesis mostly have an obstructive cause and need surgical intervention⁸. In males where there was absence of spermatogenesis along with maturation arrest or just sertoli cells visible under microscope and with proper staining can obviate unnecessary and expensive investigations in these patients⁶. In these series cytological diagnosis proved to be very accurate¹. In our present study, there was demonstrated a detailed significant correlation between the two procedures.

In 1992, with the advent of intracytoplasmic sperm injection testicular sperm retrieval in non-obstructive azoospermia has been documented^{14,15}. In the later ages, ICSI was used that offers high sperm recovery, fertilization and pregnancy rates as compared to open biopsy technique. These studies showed that a great advancement in the sperm retrieval techniques. As testicular open biopsies have cons such as inflammation, hematomas, and permanent DE vascularization of the testes that ultimately leads to testicular atrophy (Schlegel & Su, 1997). For sperm retrieval, percutaneous needle biopsy and FNA is a method of choice these days. Moreover, our study also made a comparison of sperm retrieval by open biopsy vs FNA.

Spermatozoa was obtained by Friedler from 8 of 44 patients by FNA technique with the help of 21-gauge needle while in case of open biopsy, spermatozoa was found in 18 of 4 patients. Rosenlund did comparison between FNA and open biopsy technique by extracting spermatozoa using 21-gauge needle in his 14 patients and 19-gauge needle in his 32 patients and showed that with the use of 19 gauge needle the higher number of spermatozoa can be obtained. However from his research it was cleared that FNA technique was inferior as compare to open biopsy from histological evaluation point of view. Afterwards, same group of people did reach and published article in which they claimed that using either 16 gauge or 14, the number of spermatozoa extracted were same and sufficient in both cases form histological evaluation and assessment. Moreover, they however also concluded that with a single puncture FNA may not be sufficient to represent the entire testis⁸. In our current research, 26 gauge needle was employed to study spermatozoa in 30 of 34 patients (88.8%). moreover all patients presenting with hypospermatogenesis, spermatozoa was extracted using FNA.

In some research, there is a direct relationship was found between sperm retrieval and width of needle¹³. With our current study, it was proven that with the aid of 26-gauge needle, spermatozoa of same ratio as with open

biopsy technique can be extracted. So, this proves that it is possible to extract spermatozoa even with a 26-gauge needle if aspiration done meticulously. As wider the needle, more will be complications. Histologically, with the wider needle, the number of tubular fragments tends to be higher as well as erythrocytes will be increased in number that will make microscopic evaluation an uphill task.

The incidence of intratesticular bleeding was seen in case of open biopsy as 29% and with FNA as low as 7%. In case of open biopsy, there was incidence of testis devascularization after incision by multiple attempts of the tunica albuginea as reported by Schlegel & Su⁶ and Jarrow⁷. In order to avoid the accident of ischaemic testicular damage we should avoid from multiple testicular biopsies. FNA has superiority over open biopsy in the sense that in case of FNA penetration area is way smaller as compared to knife cut (as in open biopsy) and damage rate to main artery is also way lower as compared to open biopsy. The sperm extraction rate will increase dramatically if FNA technique was performed in relation with testes anatomy and systematically as purposed by Turek⁷. By the use of technique FNA, The operator could reach to every part of testes, which makes this technique sensitive as compared to open biopsy technique as well as for sperm retrieval.

In the recent age, the European Association of Urology(EAU) provided directions with respect to infertility in males¹⁷and mentioned that testicular biopsy should be sufficient enough to provide required material and sperm for future ICSI cycles. In the fertility centers where there is availability of sperm cryopreservation, open testicular biopsy is method of choice there. However, in the areas where cryopreservation of sperms not available like in our research Centre, FNA cytology is the most recommended method of choice for examination males with azoospermia. In the cases where there is ICSI suitable, FNA and open biopsy including microsurgical are both method of choice for sperm extraction from the testicle according to EAU guidelines.

CONCLUSION

In present study, deep assessment and evaluation was done histopathologically between FNA of testes as compared with open biopsy technique. It is hence proved with support of current research that the fact that it's noninvasive, economical, have low complication rate and higher successful cases as compare to open biopsy technique.

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