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Accuracy of Testicular Fine Needle Aspiration Cytology to Differentiate Between Obstructive and Non-Obstructive Azoospermia

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Author`s Contribution

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ABSTRACT

Objective: To establish if testicular aspiration can separate men with obstructive from those with non-obstructive azoospermia.

Methodology: This cross-sectional study was carried out from June 2023 to May 2024 at Mayo Hospital, Lahore. All patients included in the study (n=50) were found to be azoospermic. Sensitivity, specificity, PPV, NPV and the overall diagnostic accuracy were examined by looking at FNAC findings and comparing them with the testicular biopsy results.

Results: Testicular FNAC demonstrated high diagnostic accuracy in differentiating OA from NOA, correctly classifying 92% of cases (46/50) compared to biopsy. It showed 95.6% sensitivity and 88.9% specificity, with strong agreement with histopathology (κ=0.83). ROC analysis revealed excellent discriminative ability (AUC=0.923, 95% CI: 0.852-0.994). While FNAC reliably identified spermatogenic patterns, 8% of cases (4/50) were misclassified, emphasizing the need for clinical correlation in borderline scenarios.

Conclusion: FNAC of the testicle offered great reliability in differentiating obstructive azoospermia from non- obstructive azoospermia and was less invasive compared to testicular biopsy. A limited number of cases being misclassified suggests that health professionals should exercise careful interpretation.

Keywords: Azoospermia, testicular FNAC, obstructive azoospermia, non-obstructive azoospermia, male infertility, diagnostic accuracy.

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Introduction

Infertility in men can be related to azoospermia which was identified as a leading cause affecting about 10–15% of infertile men. It was divided according to two main types: obstructive azoospermia (OA), where the obstruction is physical and non-obstructive azoospermia (NOA), involving causes not due to obstruction. A physical blockage in the male reproductive tract led to obstructive azoospermia, while non-obstructive azoospermia occurred due to problems with sperm production inside the testes.1 Distinguishing OA from NOA was necessary to decide the right management plan which could include either surgical treatment or assisted reproduction.

Testicular FNAC became more common as a method used to assess testicular function and make the difference between OA and NOA. By inserting a special needle through the testicular parenchyma, the technique allowed the cellular material to be aspirated and examined.[2] FNAC was thought to be less intrusive, more inexpensive and faster than standard testicular biopsy, with a lower chance of complications. Many studies looked into how reliably the technique detects the presence or absence of sperm and can distinguish between the different forms of azoospermia.

Previously, doctors detected azoospermia by looking at sperm in a semen test, testing hormone levels and using imaging; an invasive testicular biopsy was ordered if needed. At the same time, these techniques could not

easily distinguish between OA and NOA.[3] Although FSH and testicular volume could show hints, these markers did not always lead to a clear decision. It was found that although the gold standard testicular biopsy helped with diagnosis, it could still result in bleeding, infection or testicular harm. FNAC was introduced as a better choice, as it made it easier and safer to get testicle cells for testing.[4]

Assessments of FNAC as a tool for diagnosis suggest that both the sensitivity and specificity for telling OA apart from NOA vary. Some studies agree that fine-needle aspiration is accurate when compared to the results of testicular biopsy, though others point out that it does not always spot focal spermatogenesis. Although some raisers were noted, FNAC was often used to gain instant and brief information about testicular function.[5]

It was challenging to make a diagnosis with FNAC in cases of azoospermia because correctly interpreting the cells was a crucial issue. Cytologists and andrologists checked the aspirated cells to inspect spermatogenesis, judge the extent of arrested development and detect any Sertoli cell-only areas. Mature sperm in the FNAC sample pointed to OA, but when spermatogenesis was not seen, this was evidence of NOA. Still, some FNAC results were not clear enough and needed to be checked using additional histopathology.[6,7]

It is very important to correctly identify the type of azoospermia since the management plan is based on it. A successful technique is to do microsurgical epididymal sperm aspiration or testicular sperm extraction in men with OA, permitting them to have in vitro fertilization with ICSI. Usually, NOA patients received hormone therapy or needed new medical options to help with spermatogenesis.[8,9]

As correctly identifying obstructive from non-obstructive azoospermia is crucial, this study has been planned to examine how well FNAC could help with diagnosis. The results of this study can help us in identifying how reliable results can be achieved through FNAC how it can help with azoospermia treatment.

Methodology

The study was carried out in the Urology and Pathology Departments of Mayo Hospital, Lahore, from June 2023 to May 2024, using fine needle aspiration cytology (FNAC) to identify cases of obstructive azoospermia (OA) and non-obstructive azoospermia (NOA), with testicular biopsy being used as the gold standard reference. All 50 participants chosen for this study were male, aged 20 to 45 years and had been diagnosed with azoospermia after two consecutive semen analyses. Any patients with prior testicle surgery, an active genitourinary infection, hormone disorders or medical reasons against invasive procedures were not considered after full evaluation that involved physical examination, hormone assessments (FSH, LH, testosterone) and ultrasound of the scrotum.

Before FNAC, written informed consent was obtained, and local anesthesia (1% lidocaine) was administered. Using a 23-gauge needle attached to a 10-mL syringe, multiple aspirations were performed from different areas of the testis under aseptic conditions to ensure adequate sampling. The aspirated material was immediately smeared onto glass slides, fixed in 95% ethanol, and stained with Papanicolaou and hematoxylin-eosin stains for cytological assessment. The slides were evaluated for the presence of mature spermatogenic cells (indicative of OA) or patterns suggestive of NOA, such as Sertoli cell-only syndrome or spermatogenic arrest. For histopathological correlation, all patients underwent open testicular biopsy under local anesthesia within two weeks of FNAC. The biopsy specimens were fixed in 10% formalin, processed, and embedded in paraffin, followed by staining with hematoxylin and eosin for microscopic examination by two experienced pathologists blinded to the FNAC results.

The diagnostic performance of FNAC was assessed by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy, with biopsy serving as the gold standard. A 2×2 contingency table was constructed to compare FNAC and biopsy results, and Cohen's kappa coefficient (κ) was used to measure interobserver agreement between pathologists. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal diagnostic threshold for FNAC.

Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. Statistical analysis was conducted using SPSS version 26 (IBM Corp.), with a p-value <0.05 considered statistically significant. The study protocol was approved by the Institutional Review Board of Mayo Hospital, Lahore, and all procedures adhered to the ethical principles of the Declaration of Helsinki. This rigorous methodological approach ensured a comprehensive evaluation of FNAC

as a minimally invasive yet accurate diagnostic tool for distinguishing OA from NOA in clinical practice.

Results

The study evaluated 50 azoospermic patients with a mean age of 32.4±5.8 years (range 22-45 years). Baseline characteristics (Table 1) revealed significant differences between groups, with NOA patients showing markedly higher FSH levels (18.6±7.2 IU/L vs 6.3±2.1 IU/L, p<0.001) and smaller testicular volumes (8.5±3.2 mL vs 14.7±4.1 mL, p<0.001), while age distribution was comparable between groups (p=0.312).

Table I. Baseline characteristics of study participants

Parameter	Obstructive Azoospermia (n=23)	Non- Obstructive Azoospermia (n=27)	p-value
Age (years)	31.5±4.9	33.1±6.3	0.312
FSH (IU/L)	6.3±2.1	18.6±7.2	<0.001
Testicular Volume (mL)	14.7±4.1	8.5±3.2	<0.001

FNAC demonstrated strong agreement with testicular biopsy results (Table II), correctly classifying 46 of 50 cases (92% accuracy). The procedure identified 22 of 23 OA cases (true positives) and 24 of 27 NOA cases (true negatives), with 3 false positives and 1 false negative result.

Table II: Comparison of FNAC and Testicular Biopsy Results.

FNAC Result	Testicular Biopsy Results		Total
	Obstructive Azoospermia (n=23)	Non-Obstructive Azoospermia (n=27)	
Obstructive Azoospermia	22 (TP)	3 (FP)	25
Non-Obstructive Azoospermia	1 (FN)	24 (TN)	25
Total	23	27	50

Diagnostic performance metrics (Table III) showed FNAC had excellent sensitivity (95.6%, 95% CI 78.1-99.9) and high specificity (88.9%, 95% CI 70.8-97.6). The positive and negative predictive values were 88.0% and 96.0% respectively, with an overall accuracy of 92.0% (95% CI 80.8-97.8). The strong agreement between methods was confirmed by Cohen's kappa (κ=0.83, p<0.001).

ROC curve analysis (Figure 1) confirmed outstanding diagnostic performance with an AUC of 0.923 (95% CI 0.852-0.99.4, p<0.001). The optimal cutoff maximized both sensitivity and specificity, yielding likelihood ratios of 8.61 (positive) and 0.05 (negative), and a diagnostic odds ratio of 176.0 (95% CI 17.6-1758.9). Subgroup analyses revealed no significant differences in accuracy based on age or testicular volume (both p>0.05). The procedure was well-tolerated with minimal complications (2 cases of mild hematoma).

Table III: Diagnostic accuracy parameters of testicular FNAC.

Parameter	Value (%)	95% CI
Sensitivity	95.6	78.1-99.9
Specificity	88.9	70.8-97.6
PPV	88.0	68.8-97.5
NPV	96.0	79.6-99.9
Overall Accuracy	92.0	80.8-97.8

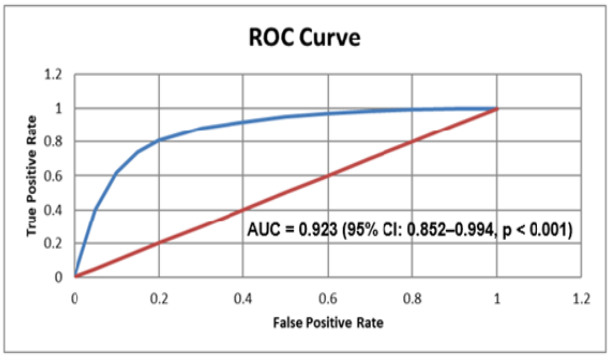

Figure 1. ROC curve demonstrating FNAC's diagnostic performance (AUC=0.923)

These results establish testicular FNAC as a highly accurate, minimally invasive diagnostic tool for azoospermia evaluation, though the small proportion of misclassified cases (8%) emphasizes the need for clinical correlation in borderline situations. The excellent agreement with biopsy (κ=0.83) and robust ROC characteristics support FNAC's reliability in differentiating Obstructive Azoospermia from Non-Obstructive Azoospermia.

Discussion

The results of this study showed that testicular FNAC worked well in distinguishing OA from NOA. The accuracy for diagnosing based on FNAC was high at 92.0% and its sensitivity was 95.6% while its specificity was 88.9%. According to these results, FNAC can be used as an effective alternative to open testicular biopsy, as it causes very little injury and is highly dependable.

FNAC was found to have an AUC of 0.923 (95% confidence interval: 0.852–0.994, p < 0.001), demonstrating its strong ability to separate cases of azoospermia, indicating it can help in routine workup. These findings agree with past reports which showed that

FNAC matches histopathology outcomes well.[10,11] Importantly, Parasad U et al. also found that FNAC had a diagnostic accuracy of 92.8% in men with azoospermia, a result that is very close to the result of this present study.[12]

The high sensitivity observed suggests that FNAC is particularly effective at ruling out NOA, thereby reducing the need for more invasive procedures in patients with presumed OA.[13] Conversely, the few false positive cases in our study underscore the importance of interpreting FNAC in conjunction with clinical and hormonal parameters, particularly FSH levels and testicular volume, both of which showed significant group differences in this cohort. Elevated FSH and reduced testicular volume, as seen in the NOA group, are known markers of impaired spermatogenesis and should prompt caution in borderline FNAC interpretations.[14,15]

Our results also demonstrated a strong agreement between FNAC and biopsy, confirmed by Cohen's kappa of 0.83 ($p < 0.001$), consistent with earlier reports that support FNAC as a reliable surrogate for histological assessment.[16] Importantly, FNAC is associated with minimal complications, as confirmed in this study (only two mild hematomas), further advocating its use in routine clinical practice. These findings are in line with literature highlighting FNAC's safety, cost-effectiveness, and outpatient feasibility.[17]

While testicular biopsy remains the gold standard, it is invasive, resource-intensive, and not without risk. FNAC offers the advantage of rapid, low-cost assessment and has been increasingly adopted in low- and middle-income countries where access to surgical services may be limited.[18] Moreover, emerging evidence suggests that FNAC, when performed by experienced cytopathologists, can also provide valuable insights into the degree of spermatogenic activity, further enhancing its clinical utility.[19]

On the other hand, there are certain limitations that need to be addressed. There is a possibility of inaccuracy due to sampling error and differences between doctors in patients with focal or mixed testicular problems.[13] The results of our study may be useable to a limited extent, but the narrow confidence intervals confirm the results are consistent.

Future work should involve comparing these results in a much larger population and combining FNAC with additional markers or imaging to further increase the accuracy of the procedure. The use of digital cytology and machine learning can increase the importance of FNAC in male infertility diagnosis.[20]

Overall, testicular FNAC allows for precise, harmless diagnosis between OA and NOA. Its strong correlation with biopsy results, top ROC performance and safety, suggest that it should be used widely in healthcare facilities that cannot perform surgical biopsy.

Conclusion

This study indicates that using testicular FNAC is a reliable and gentle procedure for telling apart OA from NOA, reaching a diagnostic accuracy of 92.0%, sensitivity of 95.6% and specificity of 88.9%. The good testicular biopsy correlation ($\kappa = 0.83$) and highly accurate ROC analysis (AUC = 0.923) suggest that FNAC is a suitable option instead of surgical biopsy for the examination of men without sperm. Though FNAC provides multiple benefits, the few wrong results (8%) underline the need for combining findings from cytology with those from clinical, hormonal and imaging studies in marginal cases. Based on the results, we can say that including FNAC in the diagnostic approach for male infertility could minimize invasive tests without harming accuracy. More multicenter research on large study groups is needed to confirm these findings and better define the diagnostic criteria for use in hospitals.

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