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Diagnostic Accuracy of Fine Needle Aspiration Cytology in Salivary Gland Lesions: A Comparative Study with Histopathology as the Reference Standard at Bahawal Victoria Hospital, Bahawalpur, Pakistan

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ABSTRACT

Fine-needle aspiration cytology (FNAC) is one of the most widely used diagnostic tools for evaluating salivary gland lesions. However, its diagnostic accuracy is uncertain due to certain factors. **Objective:** To analyze the diagnostic accuracy of FNAC in salivary gland lesions compared to histopathological examination in a clinical setting. **Methods:** This cross-sectional study was conducted from October 2021 to June 2022 at Bahawal Victoria Hospital, Bahawalpur, Pakistan. A total of 75 patients, aged 18 to 75 years, were included in the study who were previously clinically diagnosed with salivary gland lesions. Every patient included in the study underwent the FNAC, and the outcomes were compared to the histopathological analysis of the excised biopsies. The results are analyzed in terms of sensitivity, specificity, NPV, PPV, and diagnostic accuracy. **Results:** Among the 75 patients, the mean age was 42.5 ± 14.3 years, with a male-to-female ratio of 1:1.08. FNAC results showed 7 non-neoplastic lesions (9.3%), 61 benign (81.3%), and 7 malignant neoplasms (9.3%). A histopathological analysis identified 63 benign, 5 malignant, and 7 non-neoplastic neoplasms. FNAC showed a 96.0% overall diagnostic accuracy, with sensitivity, specificity, Positive predictive value (PPV), and Negative Predictive Value (NPV) of 97.0%, 88.9%,98.5%, and 80.0% respectively. Two false-negative instances (2.7%) and one false-positive case (1.3%) were found in the study. **Conclusion:** FNAC is an extremely sensitive and accurate diagnostic tool especially when it is required to distinguish between benign and malignant neoplasms. However, its low specificity increases the risk of false-negative results where histopathological confirmation becomes an integral part of the diagnosis especially the cases where clinical suspicions are high.

INTRODUCTION

Salivary gland lesions involve a wide spectrum of conditions which range from inflammatory processes to benign and malignant tumors which necessitate a reliable and accurate diagnostic tool for efficient treatment planning [1]. Fine Needle Aspiration Cytology (FNAC) is a globally preferred diagnostic method for the evaluation of salivary gland lesions as it quick, cost-effective, and minimally invasive approach [2]. In addition, FNAC is particularly useful in distinguishing non-neoplastic lesions from benign and malignant tumors. Such versatility and effectiveness of FNAC are supported by multiple studies conducted in different clinical settings [3]. The FNAC procedure is straightforward and includes aspiration of cells from the lesion through a fine-gauge needle that are then microscopically examined after some preprocessing and staining [4]. FNAC is used the most when the cytological findings are definitive enough to eliminate the need for any further invasive diagnostic procedures such

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as incisional biopsy [5]. Despite the widespread use of FNAC, there are certain limitations and the most significant is accuracy. The accuracy of FNAC can be influenced by several factors and the most significant are the experience and competency of the clinician performing the procedure, and microscopically examining the aspirate after required processing steps. The quality/ quantity of the aspiration and the inherent cytological features of the lesion also contribute to the level of accuracy [6]. Apart from the factors affecting the accuracy of the FNAC accuracy, some of the salivary gland tumors are challenging in FNAC based diagnosis due to their similarities of cytological features with benign lesions like in low-grade mucoepidermoid or acinic cell carcinoma [7]. Similarly, cystic lesions and tumors with extensive necrosis or hemorrhage may also yield insufficient or misleading cytological material which results in inaccurate results [8, 9]. The accuracy of FNAC findings can only be evaluated by comparing its results with histopathological examination results, which is an established standard for definitive diagnosis. The statistical analysis of FNAC sensitivity and specificity varies in the literature [10].

This study aimed to analyze the diagnostic accuracy of FNAC in comparison to histopathology of excision biopsies of salivary gland lesions.

METHODS

This cross-sectional study was conducted at a tertiary care hospital, Bahawal Victoria Hospital, Bahawalpur, Pakistan, from October 2021 to June 2022 after approval from IRB vide letter No. 1226/DME/QAMC Bahawalpur. A total of 75 patients were included in the study who were presented with clinically diagnosed salivary gland lesions. The inclusion criteria were patients aged 18 years and above, presenting with a well-palpable swelling in the salivary glands, suspected to be either neoplastic or non-neoplastic, who consented to undergo both FNAC and subsequent histopathological examination. On the other hand, the exclusion criteria were patients with non-palpable salivary gland lesions, with recurrent salivary gland lesions with previous diagnostic interventions and patients who were unwilling to undergo either FNAC or histopathological examination. A non-probability consecutive sampling method was employed which ensured the inclusion of all eligible patients. The sample size was calculated based on the method designed for cross-sectional studies with a prevalence of 5% (0.05) [11-13]. The sample size and prevalence are also in line with other reported studies [14, 15]. The calculated sample size is more than twice the similar study done at the Liaqat National Hospital, Karachi [16]. Given the sample size calculation, 75 patients ensured adequate power for detecting statistically significant differences between FNAC and histopathological results. FNAC was conducted on all patients using a 22-gauge needle attached to a 05 mL syringe. A written consent was taken, and vitals were measured. The needle, after creating negative pressure was inserted into the palpable lesion taking care of proper sterilization. Sufficient cellular material was aspirated by making one to two attempts at different angles. Adequacy was confirmed on-site by microscopy. Vitals were again recorded at the end of the procedure to ensure patient stability. Smears were prepared by spreading aspirated material on glass slides. Half of the slides were air dried for Diff Quik stain and the rest were fixed in 95% ethanol for Papanicolaou stain. After FNAC reporting all patients underwent surgical intervention of the lesion. The excised tissue specimens were processed, embedded in paraffin, and sectioned for histopathological examination using Hematoxylin and Eosin (H&E) staining. A histopathologist, blinded by the FNAC results, evaluated the specimens. The cytological diagnosis from FNAC was categorized into non-neoplastic, benign, and malignant neoplasms with probable differentials and further explanations in the comments. These results were then compared with the histopathological diagnosis to evaluate FNAC's accuracy. Data on patient demographics, lesion site, cytological and histopathological diagnoses, and FNAC outcomes (true positive, true negative, false positive, false negative) were meticulously recorded. Analysis of the collected data were performed using a statistical analysis tool SPSS version 26.0. The demographic characteristics of the patients were obtained based on the descriptive statistics which include mean age, gender distribution, and lesion location. In the comparison of the diagnostic results using FNAC and histopathology, the chi-square test was employed with a p-value < 0.05 considered statistically significant.

RESULTS

A total of 75 patients of age 18 to 75 years with clinically diagnosed salivary gland lesions were included in the study where the mean age of the patients was 42.5 ±14.3 years. The male-to-female ratio of the sample population was 1:1.08. The submandibular gland (n=12, 16.0%) and parotid gland (n=63, 84.0%) included the bulk of lesions among the 75 individuals. There were no cases involving sublingual or minor salivary glands. The distribution of lesions by gland location is shown in Table 1.

Table 1: Distribution of Location of Salivary Gland Lesions

Gland	Number of Cases Frequency (%)
Parotid Gland	63 (84.0%)
Submandibular Gland	12 (16.0%)
Total	75 (100%)

FNAC results categorized the lesions into three groups: non-neoplastic, benign neoplasm, and malignant

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neoplasms. Table 2 provides a comparative distribution of lesions based on FNAC and histopathological diagnoses.

Table 2: Distribution of FNAC and Histopathological Diagnosis

Diagnosis	FNAC Diagnosis (n, %)	Histopathological Diagnosis (n, %)
Non-Neoplastic	7 (9.3%)	7 (9.3%)
Benign Neoplasm	61 (81.3%)	63 (84.0%)
Malignant Neoplasm	7 (9.3%)	5 (6.7%)
Total	75 (100%)	75 (100%)

The diagnostic accuracy of FNAC in diagnosing salivary gland lesions was evaluated by comparing FNAC results with histopathological findings. The following measures were calculated:

True Positives (TP): 64 cases (85.3%) were correctly identified by FNAC as neoplastic (benign or malignant).

True Negatives (TN): 8 cases (10.7%) were correctly identified as non-neoplastic.

False Positives (FP): 1 case (1.3%) was incorrectly classified as neoplastic by FNAC.

False Negatives (FN): 2 cases (2.7%) were incorrectly classified as non-neoplastic or benign by FNAC, which were later identified as malignant by histopathology. Based on these results, the following diagnostic parameters were calculated:

Sensitivity : $\frac{64}{64+2} \times 100 = 97.0\%$

Specificity : $\frac{8}{8+1} \times 100 = 88.9\%$

Positive Predictive Value (PPV): $\frac{64}{64+1} \times 100 = 98.5\%$

Negative Predictive Value (NPV): $\frac{8}{8+2} \times 100 = 80.0\%$

Overall Diagnostic Accuracy : $\frac{64+8}{75} \times 100 = 96.0\%$

These results indicate that FNAC is highly sensitive with a sensitivity of 97.0% in the diagnosis of salivary gland lesions, with specificity of 88.9% and an overall diagnostic accuracy of 96.0%. The high PPV reflects the strong ability of FNAC to accurately identify neoplastic lesions, while the NPV suggests a moderate ability to exclude malignancy. A comparison of FNAC and histopathological findings showed a high degree of concordance between the two diagnostic methods, especially in the diagnosis of neoplastic lesions. Table 3 gives a detailed comparison of FNAC and histopathological diagnoses.

Table 3: Diagnostic Comparison of Cytology and Histopathology

FNAC Diagnosis	Histopathological Diagnosis	Concordant Cases n (%)	Discordant Cases n (%)
Non-Neoplastic	Non-Neoplastic	7 (9.3%)	1 (1.3%)
Benign Neoplasm	Benign Neoplasm	59 (78.7%)	2 (2.7%)
Malignant Neoplasm	Malignant Neoplasm	6 (8.0%)	N/A
Total	Total	72 (96.0%)	3 (4.0%)

The study identified 2 false-negative cases (2.7%) where FNAC failed to identify malignancy but later on confirmed by histopathology. Similarly, there was also a single false-positive case (1.3%), where FNAC incorrectly identified a non-neoplastic lesion as malignant. Table 4 summarizes the characteristics of the false-negative cases.

Table 4: Summary of False-Negative Cases

Case	Age (Years)	Gender	FNAC Diagnosis	Histopathological Diagnosis
1	47	Female	Benign Neoplasm	Malignant Neoplasm
2	60	Male	Non-Neoplastic	Malignant Neoplasm

DISCUSSION

This study tries to compare the diagnostic accuracy of FNAC against the histopathological diagnosis, where salivary gland lesions are under study for FNAC evaluation. As it is an inexpensive procedure that can be done in the laboratory and minimally invasive, being the primary mode of analysis, the usage of FNAC in evaluating salivary gland lesions has become widespread across all geographic boundaries. The statistical analysis of the results shows that the overall diagnostic accuracy of FNAC was 96.0%, which is highly consistent with FNAC accuracy rates reported in other similar studies. Many studies have reported diagnostic accuracy ranging from 80.0% to 95.0% [17-19]. The high sensitivity observed in this study is 97.0%, which supports the use of FNAC as a reliable diagnostic tool. The sensitivity of this study is marginally higher than the range reported in the literature, which typically falls between 87.0% and 94.0% [17-19]. Similarly, the high PPV of 98.5% indicates a strong likelihood of the correct diagnosis. The specificity of 88.9% in this study reflects its capability to correctly identify non-neoplastic lesions. Although this figure is commendable, however, is slightly lower than the specificities reported in other studies typically ranging from 85.0% to 95.0% [17-19]. The false-positive case underscores the inherent limitations of FNAC, where overlapping cytological features between benign and malignant lesions may lead to diagnostic errors. Moreover, the study identified two false-negative cases (2.7%), where FNAC failed to detect malignancies that were later confirmed by histopathology. These findings are clinically significant, as they highlight the potential risk of underdiagnosis, particularly in cases where malignant lesions present with benign-appearing cytological features and have the potential to metastasize. In situations when clinical suspicion is still high despite benign FNAC results, such false-negative results highlight the significance of keeping a high index of suspicion and the possible need for repeat FNAC or straight progression to incisional biopsy and histological investigation. Comparing current findings with those of other studies, the

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diagnostic parameters in present research are within the range of previously reported values. For example, a study reported sensitivity, specificity, and overall accuracy of 89.0%, 92.0%, and 91.0%, respectively, which are closely aligned with current results [17-19]. Other studies [20, 21] also showed high sensitivity and specificity, though slightly lower than ours, highlighting the variability across different studies due to factors such as sample size, technique, and operator expertise. In current study, the concordance rate between FNAC and histopathology was 96%, which indicates a high degree of agreement between these two diagnostic modalities in distinguishing benign from malignant lesions. This high concordance is important for clinical decision-making because it ensures the reliability of FNAC in the preoperative assessment of salivary gland lesions. The excellent sensitivity and diagnostic accuracy of FNAC shown in this work supports its use as a useful diagnostic tool for the preliminary assessment of salivary gland abnormalities [18-20]. FNAC offers a rapid and reliable method for distinguishing between neoplastic and non-neoplastic lesions, aiding in clinical management decisions, especially in the region with limited resources and a population that is not aware. However, the specificity, while high, was not perfect, indicating that FNAC should be used in conjunction with other diagnostic modalities, particularly in cases with inconclusive or suspicious results. The identification of false-negative cases highlights the importance of a cautious approach in interpreting FNAC results, especially in cases where clinical and radiological findings suggest a higher likelihood of malignancy. In such cases, a multidisciplinary approach involving repeat FNAC, imaging studies, or direct histopathological examination may be warranted to avoid diagnostic errors. While present study provides valuable insights into the diagnostic utility of FNAC in salivary gland lesions, it has its unavoidable limitations. Although the sample size is sufficient for preliminary analysis but may not fully cover the broad spectrum of salivary gland pathology due to regional and time-bound study. Furthermore, the human errors in sampling, processing, and diagnostic nature of FNAC could influence the results. Future studies with larger sample sizes and broader regional collaboration could provide more comprehensive data and further validate the findings.

CONCLUSIONS

This study effectively demonstrates FNAC as an effective diagnostic tool for the evaluation of salivary gland lesions with high sensitivity and overall diagnostic accuracy of 97.0% and 96.0% respectively. FNAC successfully identifies neoplastic lesions, particularly benign ones. The high PPV of 98.5% further supports the reliability of the FNAC's in diagnosing neoplastic conditions. A specificity of 88.9% indicates that FNAC is generally reliable, however, there is a risk of false-positive results. There were two false-negative cases, 2.7%, where malignancies were missed by FNAC but detected by histopathology evaluation.

Authors Contribution

Conceptualization: AA[1], US
Methodology: AA[1], AA, FK
Formal analysis: US, FI, AA
Writing, review and editing: FI, FK, AA,

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

The authors declare no conflict of interest.

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