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Cytohistologic Correlation Study of Thyroid Lesions with Emphasis on the Diagnostic Pitfalls

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Abstract. Fine needle aspiration of thyroid is proven to be the most economical and reliable diagnostic tool for distinguishing neoplastic from non-neoplastic nodules for better selection of patients for surgery. The aim of this study is to evaluate the diagnostic accuracy of thyroid cytology performed at our institution by correlating its results with final histopathological diagnosis. This retrospective study used two-hundred and seventy-one cases of thyroid cytology performed at King Abdulaziz University Hospital between 1995 and 2007. The corresponding histopathological slides were reviewed. The sensitivity and specificity of cytology for detecting neoplasia at our institute were calculated at 71% and 93%, respectively. The most common benign lesion diagnosed was multinodular goiter (46%), and the most common malignancy was Papillary Thyroid Carcinoma (16%). The major cause of false positive diagnosis is the overlapping cytological features in hyperplasia that can be misinterpreted as features of neoplasia; whereas the main reason of false negative diagnosis is failure to recognize the follicular variant of papillary thyroid carcinoma. The study confirmed the efficiency of cytology in the evaluation of both nodular and diffuse thyroid lesions. However; cytopathologists should be aware of the potential diagnostic pitfalls in order to achieve a higher rate of diagnostic accuracy.

Keywords: Thyroid, Fine needle aspiration, Cytology, Histology, Thyroid malignancy.

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Introduction

Thyroid nodules are common in the general population, however, only 5% of all thyroid nodules prove to be malignant[1]. Fine needle aspiration (FNA) of the thyroid was introduced in 1960 in Sweden in order to better assess the malignant character of thyroid nodules. Since then, FNA of the thyroid has gained wide acceptance and became the most non-invasive, cost-effective and efficient method for differentiating benign and malignant thyroid nodules. It has a relatively high sensitivity and specificity with a good accuracy rate approaching 90%[2-7].

Although FNA of thyroid is an accurate test with low cost and minimal complication, it has some limitations. The major one is the inability of FNA to differentiate hyperplastic nodules of goiter from benign and malignant follicular neoplasms, which unnecessarily subject such patients to surgical lobectomy for diagnostic purpose[8-9]. In addition, a proportion of cases cannot be evaluated due to paucity of material.

This study was undertaken to evaluate the cytohistological correlation between thyroid FNA and final histology diagnosis at our institute, King Abdulaziz University Hospital (KAUH), which is one of the large institutes in the western region of Saudi Arabia, for which also determines the source of diagnostic errors.

Materials and Methods

The Cytology Laboratory Archive of KAUH, between 1995 and 2007 was searched for all thyroid FNA cases. Then, the Pathology Laboratory archive was searched for follow-up surgical excision for these patients. Two hundred and seventy-one (271) cases were followed by surgery and were included in the study. The cytology slides, including cell blocks and histology slides of these cases were retrieved and reviewed for cyto-histological correlation; moreover, determine the reasons for discrepant cases.

At our institution, the thyroid FNA is performed by the surgeon for any palpable nodules, and by radiologist under ultrasound guidance for small, deep and impalpable lesions. The FNA biopsies are performed using a 22–gauge needle and sometimes a 25-guage needle attached to a 20-ml syringe. Generally speaking, three to six passes from different

parts of the nodule are obtained in order to get adequate material for interpretation. Both air-dried, diff-quick stained smears and alcohol-fixed, Papanicolaou smears are prepared. Tissue fragments aspirated into the syringe and remaining in the needle will be rinsed and fixed in 7.5% neutral-buffered formalin in order to prepare the cell blocks. The adequacy criteria used at our institution requires the presence of at least 6-8 clusters of well-fixed and well-stained follicular cells on two smears, with each cluster containing at least 10 cells[10]. Aspirates lacking this number of follicular cells can be interpreted as benign colloid nodules in the presence of abundant colloid.

The FNA cases were allocated into one of the following diagnostic groups[2,3]:

1. **Inadequate or unsatisfactory;** when the specimen did not meet the adequacy criteria described previously.
2. **Benign;** when there is no cytological evidence of malignancy.
3. **Suspicious for malignancy;** when the nuclear features and cellular arrangements are suggestive, but not conclusive, of the presence of a specific type of malignant tumor.
4. **Atypical;** when nuclear atypia such as nuclear enlargement, nuclear grooves and prominent nucleoli are present focally in the smear while background, otherwise is consistent with nodular goiter or thyroiditis.
5. **Malignant;** when cytological features are diagnostic of a specific type of thyroid cancer, including papillary carcinoma, medullary carcinoma, poorly differentiated carcinoma, anaplastic carcinoma and lymphoma.
6. **Follicular neoplasm;** when the smears are cellular with scant or absent colloid, and contain syncytial sheets or microfollicles with enlarged nuclei, coarse chromatin and prominent nucleoli.
7. **Follicular lesion;** when the smears from different passes show a spectrum of cytological features ranging from a benign nodular goiter to a possible follicular neoplasm.

Causes of false negative (FN) and false positive (FP) diagnoses in our cases were evaluated. The rates were calculated and compared to the published studies. These cases were considered to be FN or FP based on the definitions established by the Papanicolaou Society of Cytopathology[11]. A false-negative diagnosis is defined as cytologic interpretation of non-neoplastic lesion, which would otherwise, have not

had required surgical excisions, yet the resection revealed a malignant lesion. A false- positive diagnosis is defined as a cytologic diagnosis of neoplasm requiring surgical excision, but appeared to be a non-neoplastic lesion in the subsequent surgical resection. A true positive (TP) result for a neoplasm is the one with subsequent final histopathological verification for the presence of a neoplastic process. A true negative (TN) result is the one with no evidence of a neoplastic process on cytology with subsequent final histopathological confirmation. Cases with inadequate diagnosis were not included in the calculation.

Sensitivity and specificity were determined according to the following equations: Sensitivity= TP/ (TP+FN) and specificity = TN/ (TN+ FP)[11].

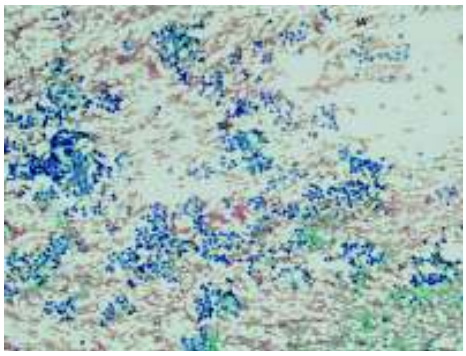
Results

A total of 922 thyroid FNAs were performed at King Abdulaziz University Hospital during the study period. Two hundred and seventy-one cases were followed by surgical excision (Table 1). The FNA slides contained adequate material for interpretation in 249 (92%) of 271 cases. Of these, 207 (83%) were from female patients, whereas the other 42 cases were from male (17%) patients; with F:M ratio of 5:1. The median age of the patients was 39 years (range 18-86). The unsatisfactory rate of the total 922 thyroid FNA performed at our institution during that period was 14%. Of these unsatisfactory cases, 22 cases had undergone surgery. The histology of these showed multinodular goiters (MNG) in 12 (55%) cases (Table 1). The total number of benign cases diagnosed by FNA was 146 (52%). The follow up of these cases confirmed the benign diagnosis in 136 (93%) of them. The majority of which turned out to be MNG (89 (61%) cases). Ten (7%) cases were called benign on the FNA which turned out to be malignant on the surgical excision; five encapsulated micro-follicular variant of PTC (Fig. 1A, B, C, D), two PTC in a background of goiter, and three minimally invasive follicular carcinomas. Five of these false negative cases were due to interpretation errors as revision of their FNA slides showed unsatisfactory specimen in one case, atypical features in one case, and follicular lesions in three cases. The remaining five cases were due to sampling errors (Table 2). Thirty-three (12%) cases were interpreted as neoplastic on FNA; 22 (67%) of these were called PTC and 11 (33%) follicular neoplasm. The

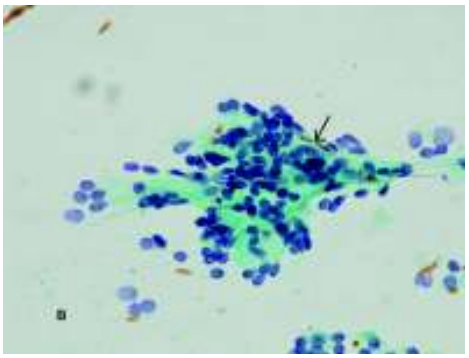
Table 1. Correlation between FNA and histological diagnosis in 271 cases.

Cytology		Histopathology	
Diagnosis	**Number (%)**	**Diagnosis**	**Number (%)**
Benign	146 (53%)	MNG	89 (61%)
		Follicular adenoma	20 (14%)
		Thyroiditis	16 (11%)
		Hyperplasia	9 (6%)
		PTC	7 (5%)
		Follicular CA	3 (2%)
		Hürthle cell adenoma	2 (1%)
Follicular lesion	56 (21%)	MNG	22 (39%)
		Follicular adenoma	15 (27%)
		PTC	8 (14%)
		Thyroiditis	6 (10%)
		Hürthle cell adenoma	2 (4%)
		Hürthle cell CA	1 (2%)
		Follicular CA	1 (2%)
		Paraganglioma	1 (2%)
PTC	22 (8%)	PTC	19 (86%)
		MNG	2 (9%)
		Medullary CA	1 (5%)
Follicular neoplasm	11 (4%)	Hyperplasia	5 (46%)
		Follicular CA	1 (27%)
		MNG	1 (9%)
		Follicular adenoma	1 (9%)
		PTC	3 (9%)
Atypical follicular cells	7 (3%)	PTC	4 (58%)
		MNG	1 (14%)
		Hyperplasia	1 (14%)
		Medullary CA	1 (14%)
Suspicious	7 (3%)	PTC	5 (72%)
		MNG	1 (14%)
		Hyperplasia	1 (14%)
Unsatisfactory	22 (8%)	MNG	12 (55%)
		Follicular adenoma	5 (23%)
		Hyperplasia	3 (14%)
		Hürthle cell adenoma	1 (4%)
		PTC	1 (4%)

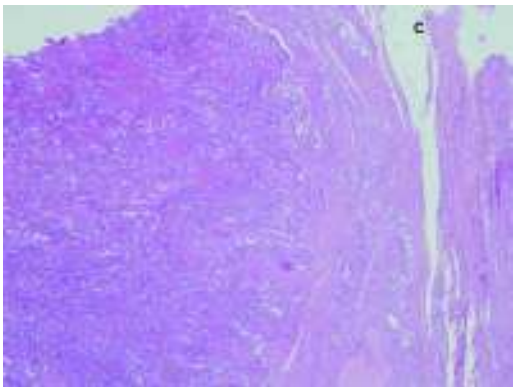
MNG = Multinodular Goiter, PTC = Papillary Thyroid Carcinoma, CA = Carcinoma



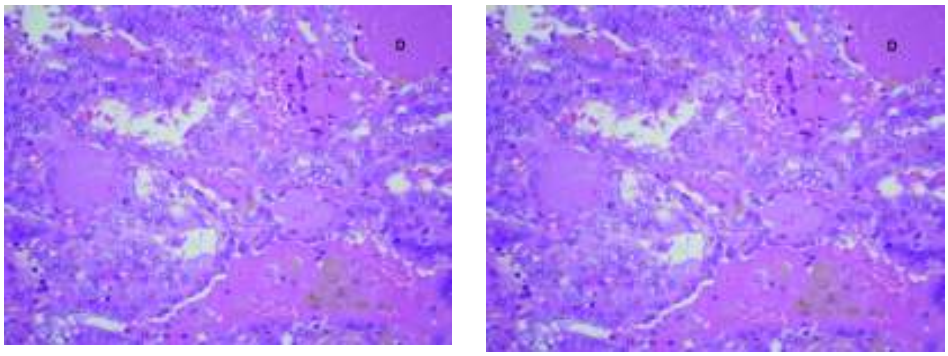
A. Cytological smear shows follicular cells arranged in a microfollicular pattern (Papanicolaou stain, 40 x).



B. High power shows nuclear enlargement and overlapping (Papanicolaou stain, 600 x).



C. Histology shows an encapsulated follicular lesion with capsular invasion (H&E stain, 40 x).



D. High power shows nuclear chromatin clearing and nuclear overlapping characteristic of papillary thyroid carcinoma (H&E stain, 600x).

Fig. 1. One of the lesions that were originally interpreted as benign and our revision showed a follicular lesion.

Table 2. Histological diagnosis of false negative cases.

Case No.	Age/Sex	FNA Diagnosis	Revised Diagnosis	Histological Diagnosis
1	35/F	Benign thyroid nodule	Same	PTC; encapsulated follicular variant
2	44/F	Benign thyroid nodule	Atypical cells	PTC; encapsulated follicular variant
3	52/F	Benign thyroid nodule	Follicular lesion	PTC; encapsulated follicular variant
4	28/F	Benign thyroid nodule	Same	PTC; encapsulated follicular variant
5	31/F	Benign thyroid nodule	Follicular lesion	Minimally invasive follicular CA
6	21/F	Benign thyroid nodule	Follicular lesion	Minimally invasive follicular CA
7	78/F	Benign thyroid nodule	Same	PTC
8	30/F	MNG	Same	PTC; encapsulated follicular variant
9	37/F	MNG	Same	Follicular CA
10	45/M	MNG	Inadequate	PTC

MNG = Multinodular Goiter, PTC = Papillary Thyroid Carcinoma

follow up of these cases confirmed the malignancy in 24 (73%) cases; most commonly (83%) PTCs, (Table 1). Ten (27%) cases were benign on the follow up (Table 3). This discrepancy is found to be due to interpretation error in 8 of the cases, in which FNA slides revision showed follicular lesion in 3 cases, benign/hyperplastic changes in 4 cases and atypical follicular cells in one case. One of the cases showed all the typical cytological features of PTC on cytology, however the total thyroidectomy showed only MNG and the carcinoma could not be found even with extensive sampling. Under the suspicious and atypical categories we had 14 (5%) cases, nine of which (64%) turned out to be PTC on the surgical excision, and 1 (7%) case as medullary carcinoma. Two (14%) cases were MNG and two cases were hyperplastic (14%).

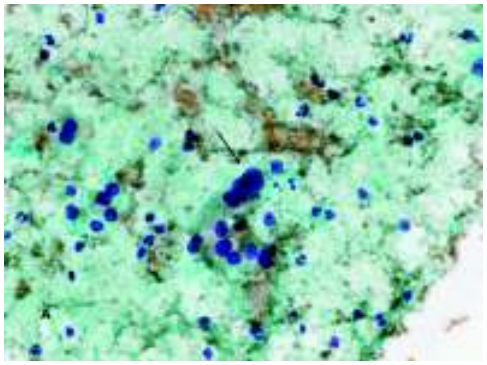
Of 148 (16%) follicular lesions on the FNA (Fig. 2A), 56 cases (38%) had undergone either total thyroidectomy or lobectomy. The histology of these showed MNG in 22 (39%) cases, follicular adenoma in 15 (27%) cases, and PTC in 8 (13%) cases. Additionally, Hashimoto's thyroiditis in 6 (9%) cases, Hürthle cell adenoma in 2 (3%) cases (Fig. 2B, 2C), Hürthle cell carcinoma in 1 (2%) case, follicular carcinoma in 1 (2%) case, and paraganglioma in 1 (2%) case.

Table 3. Histological diagnosis of false positive cases.

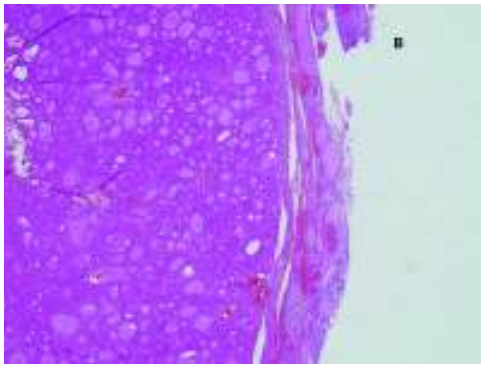
Case No.	Age/Sex	Diagnosis		
		FNA	Revised	Histological
1	45/F	FN	Hyperplastic	MNG
2	40/F	FN	Follicular lesion	MNG
3	48/M	FN	Follicular lesion	MNG
5	34/F	FN	MNG	MNG
6	18/F	FN	Same	Hyperplastic
7	22/F	FN	Hyperplastic	Hyperplastic
8	33/M	PTC	Follicular lesion	MNG
9	57/F	HCN	atypical	MNG with Hürthle cell changes
10	60/M	PTC	Same	MNG

FN = Follicular Neoplasm, PTC = Papillary Thyroid carcinoma, HCN = Hürthle cell neoplasm, MNG = Multinodular Goiter

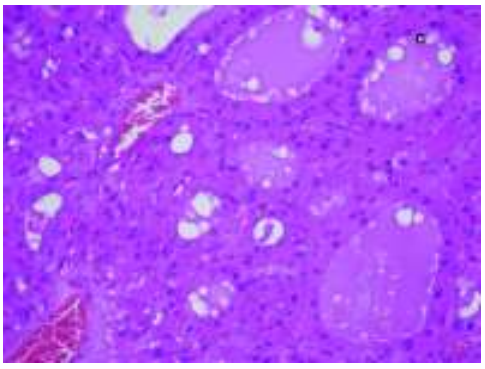
The sensitivity of the thyroid FNA at our institution was 71%, and the specificity was 93%.



A. Cytological smear showing groups of follicular cells arranged singly. The cells show mild nuclear enlargement, prominent nucleoli and rare nuclear groves. (Papanicolaou stain, 400x).



B. The follicles are lined by Hürthle cells that show mild nuclear atypia and rare grooves. This can be a source of diagnostic error on FNA (H&E stain, 600x).



C. Histology shows an encapsulated Hürthle cell adenoma (H&E stain, 40x)

Fig. 2. This case was interpreted as follicular lesion with atypia on FNA.

Discussion

FNA is the single most important diagnostic test in the initial workup of a patient with thyroid nodule[1,4,9,13]. Generally, approximately 60% of FNAs are classified as benign (range 53-90%), 4% as malignant (range 1% to 10%), 17% as insufficient sampling (range 15-20%) and 20% as follicular lesions (range 7% to 36%)[7,12]. Similar distribution of cases were present in this study in which benign cases constituted 52%, malignant 12%, follicular lesion 21 % and insufficient samples 8%. The most common benign lesion in this study was multinodular goiter 127 out of 271 (47%). Followed by follicular adenoma, 41 (15%) out of 271, while the most common malignancy was papillary thyroid carcinoma 47 out of 271 (17%). This result is similar to the previously published series, including two studies from Saudi Arabia[2-6,12,13].

In the literature, the sensitivity of thyroid FNA varies from 65 to 98% and the specificity from 73 to 100% [15]. The present study indicated the sensitivity and specificity of thyroid FNA in the lab to be 71% and 93%, respectively, which falls within the reported range. The main reason for the wide range of published sensitivity and specificity is the way the authors categorize suspicious and follicular lesions. Some authors include these in the malignant/neoplastic category, others categorize them in the negative group and still others exclude them from the calculation altogether[15]. In our study, we did not include suspicious, atypical and follicular lesion in our calculation of sensitivity and specificity.

According to the Papanicolaou Society of Cytopathology Task Force on Standards of Practice, the FN rate should not exceed 2% and FP rate should not be more than 3%[16]. These rates are based on several studies published from 1980 to 1993. However, more recent studies have reported higher and possibly more realistic rates, especially the category of FP diagnosis[2,18-23] this could be explained by the higher frequency of surgical excision of thyroid nodules after FNA. The particular increase in the FP diagnoses is most probably due to the overlapping cytological features among follicular neoplasms, hyperplastic (adenomatous) nodules, lymphocytic thyroiditis and follicular variants of PTC. The most frequent cause of FP diagnosis in our study was the interpretation error with difficulty in segregating hyperplastic nodules with microfollicular pattern from follicular neoplasms. The presence of syncytial fragments

along with microfollicular pattern strongly suggests a follicular neoplasm; however, in 15-25% of cases, the surgical excision will reveal a hyperplastic nodule[24]. Another cause of FP diagnoses in our series was misinterpretation of nuclear grooves and cytological atypia, that can be associated with goiter and hyperplastic nodules, as neoplastic. Nuclear grooving is a non-specific feature and can be seen in cases of Hashimoto's thyroiditis, nodular hyperplasia and goiter with Hürthle cell changes, follicular adenoma and Hürthle cell adenoma[6,18].

Ten false-negative cases (Table 3) with our false-negative rate of 7% were presented. This rate is consistent with recent reports in the literature that suggest an acceptable false-negative rate of 2 to 7%[20-23].

The main cause of FN diagnoses in our study was the failure to recognize the follicular variant of papillary thyroid carcinoma. Among the 10 FN cases, 7 turned out to be papillary thyroid carcinoma; five of these were of the encapsulated microfollicular variant. The difficulty in diagnosing this variant was well recognized and was due to the presence of abundant colloid, the subtle nuclear features of papillary thyroid carcinoma and the absence of papillary formations and psammomatous bodies[4,23]. The presence of atypical features such as presence of syncytial sheets, nuclear enlargement, fine chromatin pattern and nuclear grooves are important clues to identify this variant in smears. Therefore, awareness of the cytopathologist about this problem while interpreting FNAs of thyroid and the careful study of the cytological features should help in decreasing such diagnostic error.

The second reason behind our FN cases was inadequate sampling. Two cases were interpreted cytologically as goiter and on the histology in addition to the goiter, one case contained papillary carcinoma and the other contained minimally invasive follicular carcinoma. Presumed in these cases that the needle was not introduced within the thyroid nodule and what was received in FNA was the thyroid tissue adjacent to the nodule.

The indeterminate FNA is the most confusing diagnosis in thyroid cytology. The reported percentage of such diagnosis in the published studies ranges from 5 to 42%[2,7,25]. In this report, it was classified as indeterminate FNA cases into suspicious for malignancy (3%), atypical (3%) and follicular lesion (22%), which altogether accounted for 27% of

all cases of thyroid FNA included in the study. The cancer rate for the indeterminate diagnosis in this study is 72% for the suspicious, 72% for atypical diagnosis and 18 % for follicular lesion (Table 1). This high rate of malignancy warrants surgical resection of the nodules with indeterminate cytological diagnosis. Several studies have evaluated risk factors for malignancy prior to surgery. Suggested risk factors include male sex, age, nodule size and certain cytological characteristics such as nuclear appearance, cytoplasmic morphology and atypia[25,27]. Sclabas *et al*. suggested thyroidectomy for patients who are 45 years or older with a cold nodule, by thyroid scan and tumor size larger than 2 cm[3]. Cytological features in indeterminate specimens that were reported in favor of malignancy include marked crowding with three-dimensional grouping of cells, increased single cells, irregular follicles and nuclear changes, such as pleomorphism, enlargement, hyperchromasia, macronuclei and atypical mitotic figures[28]. However, such features may be present in benign nodules, especially colloid nodules with cystic degeneration or with Hürthle cell change.

The reported rate of inadequate thyroid FNA in the literature ranges from 11 to 29%[2-6,13-15]. This study showed a rate of 14%. Although thyroid FNAs are performed by clinicians rather than cytopathologists in our institution, our inadequate rate falls within expected reported range. Mandreker *et al.* found a high incidence of inadequate samples when the cytopathologist does not perform the FNA[28]. Lowhagen *et al.* have also found that the accuracy of aspiration cytology is reduced by 25% when the clinicians perform the procedure[29]. The use of ultrasound-guided FNA biopsy improves the specimen acquisition, especially in patients with small thyroid nodules or nodules that are difficult to detect on physical examination.

This study was attempted to evaluate the pattern of thyroid FNA in the western region of Saudi Arabia. The present results are comparable with the previously published data from worldwide tertiary centers and academic institutions, including data from other regions of Saudi Arabia. In conclusion, FNA of the thyroid is proven to be a reliable diagnostic tool in the initial work-up of various thyroid lesions with high accuracy rate.

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مقارنة بين التشخيص الخلوي والتشخيص النسيجي لآفات الغدة الدرقية مع التركيز على المزالق التشخيصية المحتملة

غدير مختار، و رنا بخاري

قسم علم الأمراض، كلية الطب،

جامعة الملك عبد العزيز

جدة – المملكة العربية السعودية

المستخلص. العقيدات الدرقية من الأمراض الشائعة في الممارسة السريرية. وقد ثبت علميًا أن العينة عن طريق الاختزاع الرشفي من الغدة الدرقية بواسطة إلابر هي الأداة الأكثر اقتصادًا وموثوق بها لتشخيص العقيدات الورمية المميزة من العقيدات غير الورمية، وذلك لاختيار المرضى الأفضل لإجراء عملية جراحية. والهدف من هذه الدراسة هو تقييم دقة التشخيص من الاختزاع الرشفي من الغدة الدرقية التي أجريت في مؤسستنا من خلال ربط نتائج جبعة مع تشخيص الأنسجة النهائي. تستخدم هذه الدراسة بأثر رجعي عدد ٢٧١ حالة من جبعة الغدة الدرقية أجريت في مستشفى جامعة الملك عبدالعزيز الجامعي بين عامي ١٩٩٥ و ٢٠٠٧م. تم انتشال شرائح الأنسجة المقابلة من استئصال الفص أو استئصال الدرقية واستعراضها. وتم حساب مدى حساسية ونوعية الاختزاع الرشفي من الغدة الدرقية، النتائج: إن حساسية وخصوصية الاختزاع الرشفي من الغدة الدرقية للكشف عن الأورام في معهدنا كانت ٧١٪ و ٩٣٪ على التوالي. الآفة الأكثر شيوعا هو حميدة دراق متعدد العقيدات (٤٦٪)، والورم الخبيث الأكثر شيوعا هو تشخيص سرطان الغدة

الدرقية الحليمي (١٦٪). السبب الرئيسي للتشخيص الزائف الإيجابي هو التداخل في خصائص الخلايا في تضخم الغدة الدرقية التي يمكن أن يساء تفسيرها على أنها سمات الورم، في حين أن السبب الرئيسي لدينا للتشخيص الزائف السلبي، هو عدم ملاحظة التغيرات الخلوية الناتجة عن نوع معين من السرطان الحليمي (follicular variant of PTC).

أكدت الدراسة كفاءة العينة عن طريق الاختزاع الرشفي في تقييم آفات الغدة الدرقية. ومع ذلك، ينبغي أن يكون الطبيب المشخص على بينة من المزالق المحتملة والقيود في التشخيص من أجل تحقيق أعلى معدل من دقة التشخيص.