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Cytological-Pathologic Correlation

Fine needle aspiration of non-thyroidal head and neck masses: Correlation of the cyto-histopathological diagnoses, causes of inconsistency and traps

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1. Introduction

Fine needle aspiration cytopathology (FNAC) has become a well-liked modality in the diagnosis, staging and follow-up of head and neck (H&N) masses [1,2]. Good patient acceptability, repeatability and avoidance of unnecessary surgical procedure make FNAC as the first line investigation in H&N masses [3].

This technique has a high degree of accuracy for diagnosis of both primary and metastatic diseases [4]. Correlation with subsequent tissue samples is an essential part of quality control and assurance programs in all cytopathology laboratories [5].

Since over one half of asymmetrical H&N masses in adults have been reported to be either primary or secondary malignant, an accurate diagnosis of neck swelling is of paramount importance [6]. Preoperative diagnosis of H&N lesions by FNAC offers opportunity for planning the surgical time and type of surgery, therefore a surgery for non-neoplastic entities can be avoided [7].

Clinical diagnosis of a mass in H&N region encompasses a wide spectrum of differential diagnosis. The mass may originate from salivary glands (SGs) or adjacent lymph nodes (LNs), skin appendages, soft tissues or developmental abnormalities like branchial clefts. It is particularly useful in histologically uniform neoplasms of SGs, and confirmation of LN metastases in patients with a known history of malignant tumors [7]. Most of metastatic carcinomas can be identified by their cytomorphological characteristics alone, and ancillary techniques, like immunocytochemistry, are used to overcome diagnostic difficulties [8].

It is well documented that FNAC is of value in diagnosing relapsed lymphoma [9]. FNAC has also been advocated as a useful method in comparison to more expensive surgical excisional biopsies in developing countries with limited financial and health care resources. However, the exact diagnostic accuracy of FNAC of H&N masses in comparison with the histopathological findings in three locations, namely SGs, LNs and other tissues of H&N has not been determined clearly yet.

The aim of the current work was to report the results of FNAC of SGs, LNs and other tissues of H&N in comparison to the results of histopathology, and to highlight the diagnostic accuracy and reliability of FNAC that depends solely on the cytomorphological features in H&N tissues with an emphasis on discordant cases.

2. Materials and methods

2.1. Patients

During a 10-year period in between October 2005 and October 2015, fine needle aspiration cytologies (FNAC) were performed in a total of 570 patients ranging from the age of 6 years to 94 years. The sex of patients was distributed as 250 female and 320 male. All patients with H&N masses undergone FNAC as the primary diagnostic modality and then underwent surgical biopsies were enrolled for the study. After a clinical and radiological diagnosis, FNA procedure was applied under an ultrasound guide. Exclusion criteria were the cases with inadequate FNA smears, therefore 34 (6%) of 570 patients were excluded from the study.

2.2. Cytology

The aspirations were performed by an endocrinologist and radiologist using a 25-gauge needle attached to a 10-ml syringe. The number of passes was dependent on the size of the nodule and the amount of material obtained with each pass. Three to four passes were done in the majority of cases. The smears were air-dried and stained without fixation using May-Grünwald-Giemsa staining or fixed by alcohol and stained by Papanicolaou. The number of smears ranged from one to 10 with an average of four slides per case.

In terms of reporting a description, interpretation and provisional diagnosis, the samples were graded according to the following grading system given by Draper et al. [2]. The cyto-pathological evaluation was classified as:

Grade 1: Inadequate/poorly preserved/obscured cells.
Grade 2: Benign.
Grade 3: Suspicious, probably benign.
Grade 4: Suspicious, probably malignant.
Grade 5: Malignant.

The results were categorized on the basis of the findings of the

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cytopathologist before the operation. Ancillary studies were not applied to FNA materials.

2.3. Histology

Histology included surgical excisions of lesions that were the target of cytological evaluation. These were classified and counted as 113 non-tumoral lesions, 154 benign lesions, 269 malignant. True-positive (TP), true-negative (TN), false-positive (FP), false-negative (FN) cases were identified as follows [10]:

TP: malignant cytopathology and malignant histopathology,
TN: benign cytopathology and benign histopathology,
FP: malignant cytopathology and benign histopathology,
FN: benign cytopathology and malignant histopathology.

Results of FNAC were compared with histopathology as the gold standard method. The FNAC results indicating an inconsistency/discordance were reviewed based on histopathology. Possible causes of the FPs and FNs were also investigated.

2.4. Statistical analysis

GraphPad Version 3.06 2003 program was used for statistical analysis. The Fisher's Exact Test and the Yates' Continuity Correction test (Yates' corrected Chi-square) were used to compare qualitative data. Diagnostic screening tests [sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV)], and $P < 0.001$, $P < 0.01$ and $P < 0.05$ levels were considered significant.

3. Results

The study comprised 570 patients, separately 310 (54.4%) men and 260 (45.6%) women. As shown in Fig. 1, 5.9% of specimens did not allow an adequate cyto-diagnosis and excluded from the study. Out of 536 cases, 21.1% had non-tumoral lesions, 28.7% had benign lesions. Almost half of all H&N lumps were (50.2%) malignant.

Among 232 studied cases with cytopathology of SGs, the most frequent diagnosis was benign lesion (53.8%) and the least one was inadequate samples (6.9%). Histopathological examination of SGs showed that most of the cases were benign-neoplastic (Fig. 2) and malignant tumors (Fig. 3, Table 1). Pleomorphic adenoma (PA) was the most common diagnosis among the benign neoplasms with a ratio of 64.7%. The cases with cervical lymphadenopathy were diagnosed generally with suspicious lesions (50.2%). A total of 151 cases with 'suspicious' diagnosis in FNAC had lesions mostly located in the lymph nodes (74.8%) (Table 2). On the other hand, lymphoma (61.3%) was the most common type of cancer among malign LN cases, followed by metastatic carcinomas (41.6%) (Fig. 4).

In terms of FNAC diagnosis of other locations in H&N, there were mostly malignant tumors 37.6% of patients. Histopathology of these lesions showed that the cases were mainly malignant tumors (56.7%) (Table 1).

3.1. Comparison of cyto-histopathological findings of FNAC in various locations

Of a total of 536 cases of FNAC, 9.9% had FP diagnosis and 3.2% had FN diagnosis (Table 3) with a statistical significant difference between diagnosis ($P < 0.001$). Moreover, the results of FNAC for SGs and LNs were found statistically different among the diagnosis ($P < 0.0001$ and $P < 0.05$, respectively), but not in other lesions in H&N ($P = 1.00$).

The reasons of FP diagnosis in SGs were the presence of atypical squamous metaplastic cells (SMCs) with narrow cytoplasm and extensive necrotic background, few/none myoepithelial cells, hypocellular smear, plenty of acinic and ductal cells or the absence of chondromyxoid matrix and oncocytic cells, and a whole clinical history of patient. Also, "necrotizing sialometaplasia" and "sclerosing policystic adenosis" resulted in mis-diagnosis and over-diagnosis of SG cases.

Due to the course of FNA technique, degenerated lymphoid cell, histiocytes, Hodgkin-like cells, eosinophilic leukocytes which can be seen in Hodgkin's lymphoma (HL), numerous centroblast-like large, mitotic cells, and uniformly shaped lymphocytes with small/medium-sized nuclei, granular chromatin, and no obvious granulomatous structure, many FP diagnoses were noted in histopathology of LNs. Another reasons of FP were small degenerated lymphoid groups due to smearing technique of FNAC, misinterpreted centroblasts and centrocytes based on the sampling of germinal and paracortical areas, and sampling from LN in parotid gland (PG).

Considering the lesions in other H&E regions, the large number of atypical SMCs, FNA performed from normal SG tissues instead of the cystic lesion, degenerated lining epithelial cells exfoliated into the lumen of cysts resulted in FP diagnosis.

Presenting the reasons of FN in diagnosis of SGs, no chondromyxoid substance, sampling error in FNA, and rare epithelial cell groups were found. In the smears of LNs, the presence of many phagocytic macrophages (tingible bodies) and epitheloid histiocytes, polymorphic

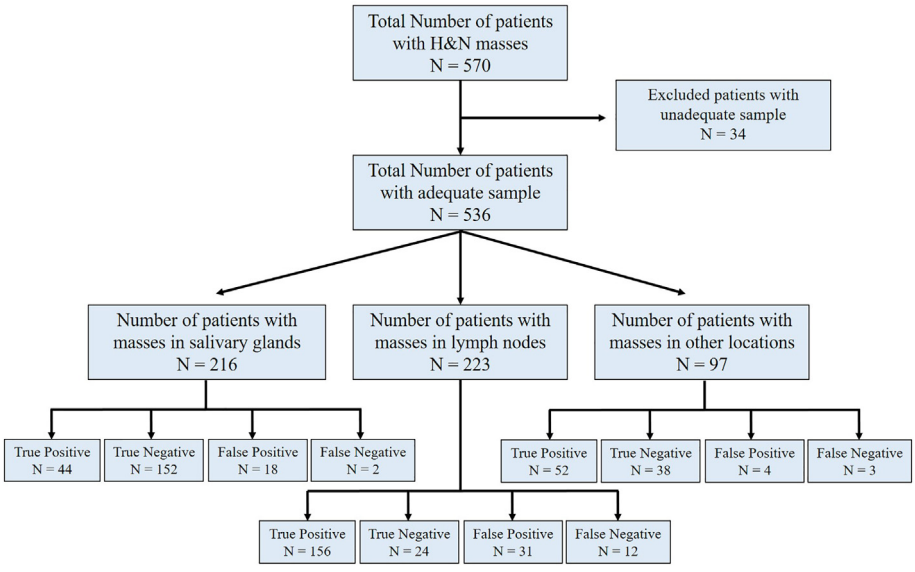

Fig. 1. A diagram showing the selection of patients.

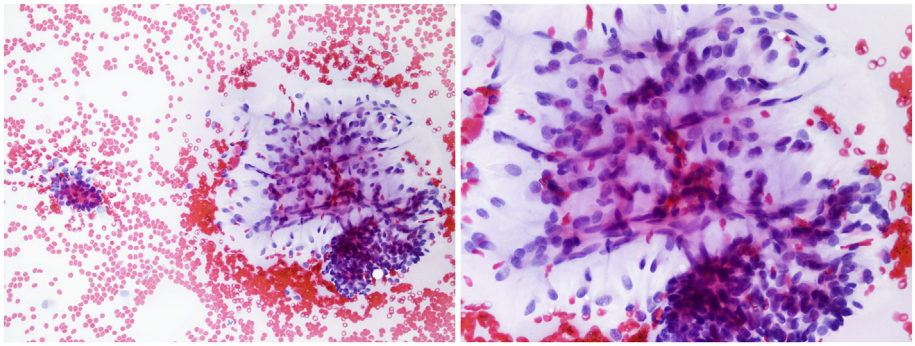

Fig. 2. Fine needle aspiration cytology of pleomorphic adenoma of salivary gland (Papanicolaou, left: ×200, right: ×400).

lymphoid and hemorrhagic background, very rare atypical cells, aspirations from the patient with a clinical abscess history, sampling from reactive zone or submandibular area, hypocellular smears due to inadequate sampling led to FN diagnosis.

3.2. Diagnostic sensitivity, specificity, accuracy and predictive values of FNAC

Overall, FNAC had sensitivity of 93.7%, specificity of 80.2%, accuracy of 86.9%, PPV of 82.6%, and NPV of 92.6% in diagnosis of H&N malignancies (Table 4). Of note, SG cases in FNAC were observed to reveal the highest sensitivity (95.7%) and NPV (98.7%) with a dramatic significance level of $P < 0.0001$. FNAC of other H&N tissues showed the best results for diagnostic accuracy (92.8%), specificity (90.5%) and PPV (92.9%) with significance levels of $P < 0.001$, $P < 0.01$ and $P < 0.01$, respectively.

4. Discussion

Assessing the effectiveness of FNAC in investigation of H&N masses, we carried a comprehensive 10-year retrospective review of our FNAC experiences in our institution. The quality control measurements in our overall FNAC experience for non-thyroidal H&N masses are in line with the literature. FNAC of SG cases revealed the highest intra-class significance, sensitivity and NPV. FNAC of other H&N cases showed the highest specificity, accuracy and PPV.

It becomes a general opinion that FNAC is recommended in the initial assessment of a palpable H&N mass [11,12]. However, cytodiagnosis and histopathology should always be considered in the context of clinical findings as a part of overall evaluation of H&N lesions. Thus, in the present, it was aimed to compare cytopathological and histopathological findings of H&N masses in our Clinique to determine the diagnostic accuracy of FNAC.

In the literature, a meta-analysis by Tandon et al. reported a raw data (3459 FNAs) for FNAC of H&N with a sensitivity of 89.6%; specificity of 96.5%; FP rate of 3.5%; FN rate of 10.3%; and accuracy of 93.1% [11]. The PPV was 96.2% and the NPV was 90.3%. However, the study did not reported any distinction between clinical follow-up and histopathology as the method of verifying FNAC results. Data relating to a non-diagnostic FNAC was also confusing. The range of non-diagnostic aspirates was wide, ranging from 3 to 30% [11]. Moreover, in a number of papers, there was no clear statement regarding non-

Table 1
Cyto-histopathology findings of all non-thyroidal H&N cases.

Location	N (%)	Cytopathological diagnosis	Histopathological diagnosis
Salivary glands	Non-tumoral	29 (12.5)	22 (10.2)
	Benign	125 (53.8)	148 (68.5)
	Malignant	41 (17.7)	46 (21.3)
	Suspicious	21 (9.1)	–
	Inadequate	16 (6.9)	–
	Total	232	216
Lymph node	Non-tumoral	34 (14.9)	55 (24.7)
	Benign	2 (0.9)	–
	Malignant	72 (31.4)	168 (75.3)
	Suspicious	115 (50.2)	–
	Inadequate	6 (2.6)	–
	Total	229	223
Others[a]	Non-tumoral	36 (33.0)	36 (37.1)
	Benign	5 (4.6)	6 (6.2)
	Malignant	41 (37.6)	55 (56.7)
	Suspicious	15 (13.8)	–
	Inadequate	12 (11.0)	–
	Total	109	97
Total	Non-tumoral	99 (17.4)	113 (21.1)
	Benign	132 (23.2)	154 (28.7)
	Malignant	154 (27.0)	269 (50.2)
	Suspicious	151 (26.5)	–
	Inadequate	34 (5.9)	–
	Total	570	536

[a] Cystic lesions (branchial or thyroglossal), primary squamous cell carcinoma, nasopharyngeal carcinoma, malign melanoma, mesenchymal tumors.

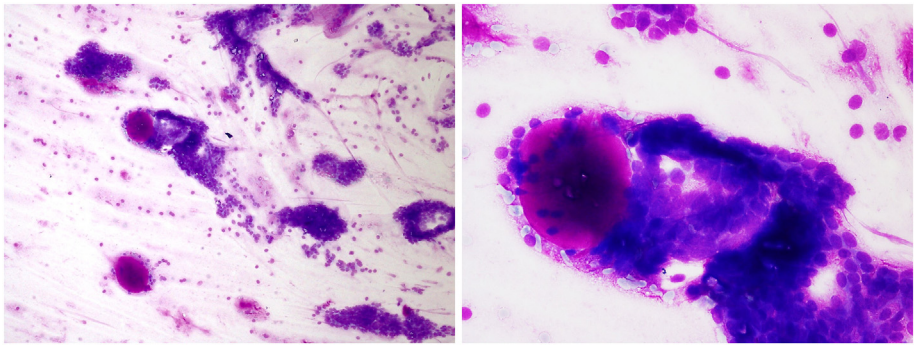

Fig. 3. Fine needle aspiration cytology of adenoid cystic carcinoma (May-Grünwald-Giemsa, left: ×100 right: ×400).

Table 2
The distribution of histopathological diagnoses of the lesions in H&N masses according to the location of patients with 'suspicious' diagnosis in fine needle aspiration cytology.

Number of cases (%)	Localization			
	Salivary gland (%)	Lymph nodes (%)	Others[a] (%)	Total
Non-tumoral	2 (9.5)	29 (25.2)	2 (13.3)	33 (21.9)
Benign neoplazm	13 (61.9)	0 (0.0)	1 (6.7)	14 (9.3)
Malign neoplazm	6 (28.6)	86 (74.8)	12 (80.0)	104 (68.9)
Total	21	115	15	151 (100)

[a] Cystic lesions (Branchial or thyroglossal), Primary squamous cell carcinoma, Nasopharyngeal carcinoma, Malign melanoma, Mesenchymal tumors.

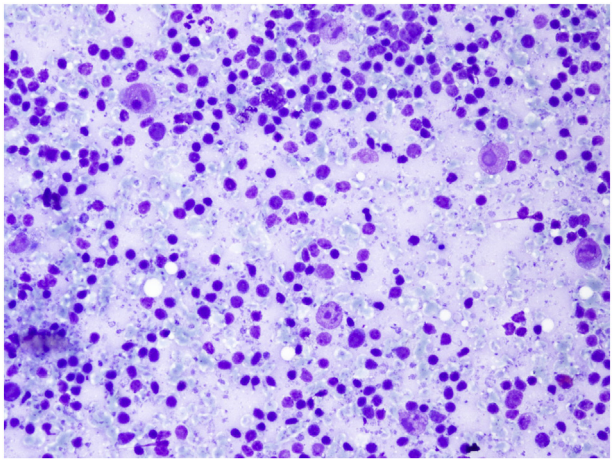

Fig. 4. Fine needle aspiration cytology of Hodgkin lymphoma of cervical lymph node (mixed cellular type) (May-Grünwald-Giemsa, ×400).

Table 3
Cyto-histopathologic findings of fine needle aspiration cases in different localizations.

Number of cases (%)	Localization			
	Salivary gland	Lymph nodes	Others[a]	Total
True positive	44 (20.4)	156 (67.0)	52 (53.6)	252 (47.0)
True negative	152 (70.4)	24 (10.8)	38 (39.2)	214 (39.9)
False positive	18 (8.3)	31 (13.9)	4 (4.1)	53 (9.9)
False negative	2 (0.9)	12 (5.4)	3 (3.1)	17 (3.2)
Total	216	223	97	536
P *value*	***< 0.0001*** ***	***0.0351*** *	1.000	***0.0007*** ***
Inadequate	16 (6.9)	6 (2.6)	12 (11.0)	34 (6.0)
Total	232	229	109	570

[a] Cystic lesions (branchial or thyroglossal), primary squamous cell carcinoma, nasopharyngeal carcinoma, malign melanoma, mesenchymal tumors.
* $P < 0.05$ vs all groups.
*** $P < 0.001$ vs all groups.

diagnostic aspirates, and were either not mentioned at all or may have been included into the analysis. On the contrary, the present study reported a higher sensitivity as 93.7%, higher NPV as 92.6% and lower FN ratio as 3.2 than the findings of Tandon et al., as well as 6% of inadequacy rate; and outcomes of these 'inadequate' aspirates were also investigated by histopathological follow-up.

As noted by Orell, FNAC can be used in stepwise manner, initially to determine SG origin of a neck swelling; secondly to offer guidance to its neoplastic or non-neoplastic nature; and finally, in case of tumors, to define its potential benign or malignant character. The first is worthy of emphasis, as it may be difficult or impossible to distinguish SG lesions from enlarged cervical LNs, cysts, adnexal lesions, or soft-tissue swellings clinically [13]. In this retrospective study, we could not analyze the provisional clinical diagnoses in detail prior to FNA, but it was frequently noted in request forms that patients were suspected of having lymphadenopathy, particularly in submandibular area and parotid tail.

The sensitivity of FNAC for the diagnosis of malignant neoplasms of SGs, ranged from 55 to 98% in the literature [14]. In the present study the sensitivity of FNAC of SGs was as high as 95.7%. FNAC of SG tumors that show uniform histology throughout the lesion has proven to be a reliable and valuable technique for evaluation. On the other hand, neoplasms with a variety of histologic patterns and cell types provide a source of misdiagnosis related to sampling in FNAC. The proportion between epithelium and chondromyxoid stroma, variations in the appearance of epithelial cells, hyaline structures present in the stroma should be considered PA diagnosis [10,14]. In the diagnosis of adenoid cystic carcinoma (ACC), structures similar to hyaline globules may be seen occasionally in non-SG tumors [15]. A meta-analysis of the large series showed that 84.1% of ACCs were correctly diagnosed or suspected by FNAC [15]. 94.1% of ACCs (n = 17) were correctly cytodiagnosed in the present study (data not shown), with a higher ratio than the literature.

Classically, cytopathological components of Warthin tumor (WT) are oncocytic cells in clusters or in papillary structures, lymphoid stroma resembling stimulated LN, and focal necrosis [16] Metaplastic squamous cells with dirty background raises the possibility of metastatic squamous cell carcinoma (SCC). We have experienced this trap in three cases in ten years; hypercellular smears of the patient were misdiagnosed as 'papillary thyroid carcinoma (PTC) metastasis' in FNAC but as 'WT' in excisional biopsy. The cystic nature, as well as mucinous or metaplastic squamous cells, can also be difficult to differentiate from low-grade mucoepidermoid carcinoma (MEC) [10,17]. We were also unable to discriminate subtype 3 cases of MEC correctly, defined these patients as abscess formation.

As an average of 5–10 LNs are usually located in each PG, various reactive and neoplastic lymphoid lesions may manifest with PG enlargement [18,19]. Four of five lymphoma cases in our study were observed in PG and one case was in minor SG.

FNAC of H&N masses in LNs has considerable value in differentiation of H&N lymphoma from non-lymphoma etiologies; and is recommended as a screening test for the diagnosis of H&N lymphoma [1]. While primary diagnosis of lymphoma by FNA is generally not considered definitive; it is helpful in clarifying nature of the process and the direction of additional diagnostic tests. Flow cytometric immunophenotyping (FCI) and immunohistochemistry are particularly useful [20]. Although the gold standard method for diagnosis of lymphomatous LN lesions is histopathology, FNAC in conjunction with FCI has become a reliable and accurate method for diagnosis and classification of many lymphoproliferative disorders as well as to recognize the residual and recurrent lymphoid malignancies [21]. As the modern classification of World Health Organization (WHO) is based on morphology, immunology, and cytogenetic changes, FNAC with FCI has shown high sensitivity and specificity in diagnosis of LN lesions, especially NHL [22,23]. Among 168 malign cases of LNs in this study, 72 patients were diagnosed with malignant tumor in FNAC correctly so this can be considered a low diagnostic accuracy for FNAC of LNs.

HL is diagnosed in FNAC based on the demonstration of Hodgkin (H) and Reed-Sternberg (RS) cells in a background of appropriate reactive cellular components [24]. But one of the well-known pitfalls in FNAC in diagnosis of HL is the challenge to identify the rare or absent classical H-RS cells. In fact, HL accounts for the majority of FNs in FNAC of malignant lymphoma. The diagnosis of nodular sclerosing classical HL (NSCHL), in particular, has historically been challenging due to hypocellular smears as a result of marked LN fibrosis that characterizes this variant of HL. We experienced the same difficulty in our 11 NSCHL cases correctly diagnosed in this study. Extremely careful microscopic

Table 4
The statistical correlation results in detecting the diagnostic sensitivity, specificity, accuracy and predictive values of fine needle aspiration cytology.

Localization	Diagnostic scan				
	Sensitivity	Specificity	Accuracy	PPV	NPV
Salivary glands	95.7%	89.4%	90.7%	71.0%	98.7%
Lymph nodes	92.9%	43.6%	80.7%	83.4%	66.7%
Others[a]	94.6%	90.5%	92.8%	92.9%	92.7%
P value	*0.75*	***< 0.0001******	***0.0013*****	***0.0016*****	***< 0.0001******
Total	93.7%	80.2%	86.9%	82.6%	92.6%

PPV: positive predictive value.
NPV: negative predictive value.
CI: confidence interval.
LR: likelihood ratio.
[a] Cystic lesions (branchial or thyroglossal), primary squamous cell carcinoma, nasopharyngeal carcinoma, malign melanoma, mesenchymal tumors.
** $P < 0.01$ vs all groups.
*** $P < 0.001$ vs all groups.

screening is crucial to recognize rare H-RS cells and to differentiate them from similar appearing reactive mimickers, like immunoblasts, histiocytes, and fibroblasts [25,26]. H-RS cells may sometimes be mistaken as immunoblasts, particularly in viral lymphadenitis [27]. Uncommonly, HL can present with clinical and cytopathologic features mimicking an infectious etiology. The suppurative background may lead to misinterpretation of true rare H-RS cells for reactive immunoblasts, histiocytes, or fibroblasts [25]. RS-like cells have been also recognized in certain NHL subtypes, metastatic melanoma, metastatic carcinoma, and reactive hyperplasia of LN [24], as observed in the present study.

Contributing factors for a FN diagnosis include obscuring reactive inflammatory cells, fibrosis of the involved LNs, and partial involvement of LN by HL, sampling error, and misinterpretation. The reported accuracy for diagnosis of HL by FNAC varies between 30 and 92% in the literature [27]. In this study, 92.3% (n = 24) of 27 biopsy-proven HL cases were correctly diagnosed or strongly suspected based on the FNAC findings. Collections of histiocytes mimicking poorly formed granulomas were present in our FN cases.

In the present study, in case of clinical and cytopathological findings of inconsistency in diagnosis of malignancy, FNAC of LN cases were reported as, “suspicious/atypical lymphoproliferative lesion”; followed by the comment of “Cytopathological findings are suspicious for malignant LN involvement. Histopathological and immunohistochemical evaluation is recommended with an excisional biopsy for the definitive diagnosis and subtyping.” This appeared to be an effective approach to guide patients to excisional biopsy. Also, one of future goals should be enlarging the scope of this study with new FCI of H&N masses, especially in LNs.

SCC is the most common carcinoma encountered in H&N area. Several diagnostic problems and pitfalls that might be seen in FNAC of SCC, include cystic changes, well-differentiated SCC, spindled SCC, and SCC with foreign-body giant cells, keratin plaques, and ghost cells. In cystic SCC, if there are abundant histiocytes and debris in the background suggestive of cystic contents and unequivocal malignant squamous cells are not present, a careful search for any nuclear atypia and abnormal keratinization helps to avoid FN misdiagnosis on cytopathology [28,29]. In particular, the most common metastatic neoplasms involving the cervical LN chain are SCC followed by nasopharyngeal carcinoma, papillary thyroid carcinoma and malignant melanoma. After age 40, SCC becomes the primary diagnostic consideration, and after age 60, SCC is the single most important entity in differential diagnosis [29]. One of the SCC cases in the present series were older than 40 years of age and the average age was 69.9 years. 42.9% of 70 SCC cases in the present series was correctly diagnosed in LNs while 28 out of 31 (90.3%) SCC cases in other tissues of H&N were correctly cyto-diagnosed.

Discrepancies in FNAC of H&N cases may still occur in spite of careful examinations. However, an optimal FNA technique to get a proper sample, onsite adequacy evaluation in selected cases, and triage by a pathologist experienced in cytopathology, as well as efforts in applying all cytodiagnostic criteria, and clinical, radiologic, and intradepartmental consultations when appropriate would help decrease the incidence of misdiagnosis. Last but not the least, cytodiagnosis should always be considered in the context of clinical findings, correlated with histopathological findings.

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Declarations of interest

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