Touch Imprint Preparations And The Diagnosis of The Head And Neck Mass lesions: Comparative Study

Mahmoud R. Hussein, Usama M. Rashad; and Kamal Abdulall Alsherkawy

Background: Touch imprint cytology (TIC) is a method used with remarkable success in the diagnosis of tumors. Objectives: To assess the utility (sensitivity, specificity and accuracy) of an intraoperative touch imprint (TIC) in the diagnosis of the ear, nose and throat and head and neck mass lesions, we compared TIC with permanent hematoxylin and eosin histology in the diagnosis of these lesions. Materials and methods: We evaluated thirty ear, nose and throat and head and neck mass lesions by TIC. The results of TIC preparations were intraoperatively correlated with gross picture and subsequently with permanent histologic sections of representative biopsies. The study period ran between January, 2003, and January, 2004. Eight touch imprints and histology sections were prepared from every case and were reviewed by two pathologists independently. Further immunohistochemical evaluation was performed on the tissue sections.
Results: Examination of TIC identified 12, 16 and 2 specimens as benign, malignant and suspicious of malignancy respectively. Permanent histological sections revealed 15 benign and malignant cases (each). There was one false-positive case. Sensitivity and specificity of touch preparation cytology were 88% and 92%, respectively. Conclusions: TIC can rapidly and reliably evaluates ear, nose and throat and head and neck mass lesions and therefore overcome sampling errors and artifacts that may be related to frozen section evaluation.

Lecturer, Pathology Dept, Faculty of Medicine, Assiut University
USAMA RASHAD
Lecturer, ENT Dept, Faculty of Medicine, Assiut University
KAMAL ABDULALL ALSHERKAWY¶
Lecturer, Surgery Dept, Faculty of Medicine, South Valley University

THE routine management of the ear, nose and throat and head and neck mass lesions entails histological examination of the initial frozen sections, and then followed by the evaluation of permanent Formalin fixed paraffin embedded tissues. Such a process is worrying for the surgeon who is unsure about the tentative diagnosis of the frozen sections that might have mistakes. Moreover, the pathologist is required to make a hurried diagnosis on specimens that have been suboptimaly prepared. Furthermore, the use of frozen section is problematic for the operating room staffs, who are uncertain whether a procedure will last for 15 or 115 minutes. Therefore, reliance on frozen section diagnosis has been rendered increasingly obsolete (Chao et al., 1996 and Helpap and Tschubel, 1977).

During the past two decades, cytological preparations emerge as routine methods for diagnosis in pathology practice. This trend is due in part to improved methods for materials harvesting and processing as well as excellent radiological methods. The value of these cytological preparations stems from the fact that they allow the pathologist to render a quick, preliminary diagnosis before the final histological examination. Of note, diagnosis from histological preparations may be delayed due mainly to time taken for processing of the biopsy (Helpap and Tschubel, 1977).

Ear, nose and throat and head and neck mass lesions encompass a wide variety of inflammatory and neoplastic lesions (Chao et al., 1996 and Batsakis et al., 1981). The specificity and sensitivity of the different methods for diagnosis of ear, nose and throat and head and neck mass lesions have been subject of divergent views. This controversy may in part be explained by variation in the number of specimens reviewed, the staining methods, and the extent of involvement by the neoplastic process, the presence or absence of fibrosis and/or necrosis in the biopsy sections. This controversy stresses the complementary roles of

the cytology preparations and biopsy for detection of malignancy. One of these cytological preparations is the touch imprint cytology (TIC) which represents a simple, rapid (less than five minutes), reliable, and highly sensitive procedure for evaluation of the inflammatory and neoplastic lesions. To date, and to the best of our knowledge, only few reports are available about the usefulness of TIC in the diagnosis of ear, nose and throat and head and neck mass lesions (Florell et al., 2001, Hahn et al., 1995 and Gentry 1986).

To fill this gap in the literature we carried out the present study. We planned to investigate the reliability of the touch imprint preparation, and the reproducibility of its results. To achieve this goal, we examined thirty mass lesions from different sites of the ear, nose and throat and head and neck. The lesions were examined using: 1) touch imprint preparation, 2) conventional histology and 3) immunohistochemistry. The diagnostic yields have been compared among these different methods to determine the sensitivity, specificity and the accuracy of TIC in the evaluation of the head and neck mass lesions.

Materials and Methods:

Tissue specimens: Tissue samples were obtained from thirty patients presented by ear, nose and throat and head and neck mass lesions (namely from the nose, larynx, nasopharynx, oropharynx, hypopharynx, thyroid, parotid and lymph nodes). Endoscopy under general anaesthesis was needed for all laryngopharyngeal and nasal lesions. Detailed history, clinical examination and pre-operative investigations were performed. A summary of clinical characteristics is presented in Table 1.

Touch imprint preparation: Immediately after obtaining the biopsy specimens, and prior to placing them in fixative, each specimen was imprinted on several glass slides (eight slides) for cytologic study. Some imprints were fixed immediately and stained with hematoxyline and eosin stain.

Cytological examination: The cytological results were reported as: 1) malignant (the cellular findings are diagnostic of malignancy i.e. featuring nucleomegaly, hyperchromatism, pleomorphism, neocleomegally, and irregularity of the nuclear borders), 2) suspicious for malignancy (suggestion of cancer but uncertain due to limited number of cells or to degree of atypia), 3) negative for malignancy (no evidence of malignancy), and 4) unsatisfactory specimen (scant cellularity, air drying or distortion artifact, obscuring blood or inflammation).

Histological and cytochemical evaluations: The biopsies were Formalin fixed, and following fixation, the specimens were processed for routine histology and immunohistochemistry. The results obtained by TIC were compared with the histological diagnosis of surgical biopsies.

Immunohistochemical analysis: Immunostaining was done to confirm the diagnosis of undifferentiated malignancies (poorly differentiated carcinomas and lymphomas) Immunostaining was carried out as previously described (Hussein et al., 2002). Briefly, antigens were retrieved by microwave (0.01 mol/L citric acid buffer, pH 6.0 for 10 minutes). Non-specific protein binding was blocked with 10-min. exposure to 10% normal goat serum. Sections were incubated with mouse monoclonal antibodies for 30 min at 37^0C (Clone 124, Clone L26, and Clone PC3 for bcl-2, CD20 and CD3 respectively, obtained from DAKO Corp, CA,USA). A catalyzed signal amplification system (K1500, DAKO) according to the manufacturer instructions. The slides were independently evaluated by two observers.

Negative controls: Additional sections, running in parallel but with omission of the primary antibody served as the negative controls (Hussein et al., 2002).

Positive controls: Specimens consisted of reactive lymphoid hyperplasia (for Bcl-2, CD20 and CD3), and squamous carcinomas (for Cytokeratin) were used as positive controls.

Evaluation of bcl-2, Cytokeratin CD20 and CD3 staining: The presence of cells with clear membranous staining identified CD20 and CD3 positive cells. Alternatively, expression of Bcl-2 and cytokeratin proteins was identified as diffuse golden yellow cytoplasmic staining (sites of the mitochondria and intermediate filaments respectively).

Statistical analysis: Statistical analysis was done using Statistix for Windows. Analytical Software Program. The sensitivity, specificity, and the predictive values were calculated.

Results

The study period ran between January 1, 2003, and January 1, 2004. Eight histology sections and eight touch imprints were prepared from every case and were reviewed by two pathologists independently. The cytological interpretation was carried out intraoperatively. Histological examination of the permanent sections was carried several days later. Initially, the cytological evaluation of the TIC revealed 12, 16, and 2 cases as benign, malignant, and suspicious for malignancy. No unsatisfactory cases were seen. Alternatively, further histological examination of the permanent histological sections revealed fifteen cases each as malignant and benign lesions. The concordance between touch imprint and paraffin sections was 90% (27 of 30). The sensitivity and specificity of TIC preparations in detecting malignancy were 88% and 92% respectively. The positive and negative predictive values were 100% and 50% respectively (Tables 1 and 2).

Table 1: Clinical characteristics of patients with head and neck mass lesions: Sq.C.C., squamous cell carcinoma; NHL, non - Hodgkin's lymphoma and T.B, Tuberculosis.

Case	Age	Sex	Sit of the lesions	TIC	Biopsy
1	60	F	Nasopharyngeal mass	Positive	Sq.C.C.
2	70	M	Supraglottic mass	Positive	Sq.C.C.
3	65	M	Transglottic mass	Positive	Sq.C.C.
4	54	M	Nasopharyngeal mass	Suspicious	Dysplasia
5	51	M	Vallicular mass	Positive	NHL
6	38	M	Cordal mass	Positive	Sq.C.C.
7	15	M	Mass in the nasal cavity	Negative	Polyp
8	30	F	Nasopharyngeal mass	Positive	NHL
9	17	F	Postcricoid mass	Positive	Sq.C.C.
10	80	F	Postcricoid mass	Positive	Sq. C.C.
11	60	F	Oropharyngeal mass	Negative	Inflammation
12	30	F	Pyriform fossa mass	Positive	Sq.C.C.
13	53	M	Postcricoid mass	Positive	Sq.C.C.
14	25	M	Tonsillar mass	Positive	NHL
15	50	F	Postcricoid mass	Positive	Sq.C.C.
16	30	F	mass in the pyriform fossa	Suspicious	Sq.C.C.
17	24	M	Nasopharyngeal mass	Negative	Polyp
18	50	F	Thyroid mass	Negative	Goiter
19	50	F	Scalp mass	Positive	Sq.C.C.
20	36	F	Thyroid mass	Positive	Adenoma
21	35	M	Cervical mass	Negative	T.B
22	50	M	Post auricular mass	Negative	Inflammation
23	-	F	Thyroid mass	Negative	Goiter
24	-	F	Cervical mass, lymph node	Negative	Inflammation
25	-	F	Parotid mass	Positive	Sq.C.C.
26	-	M	Thyroid mass	Negative	Goiter
27	-	F	Parotid mass	Positive	Adenoma
28	37	M	Submandibular mass	Negative	Inflammation
29	65	F	Thyroid mass	Negative	Goiter
30	37	F	Cervical mass, lymph node	Negative	T.B

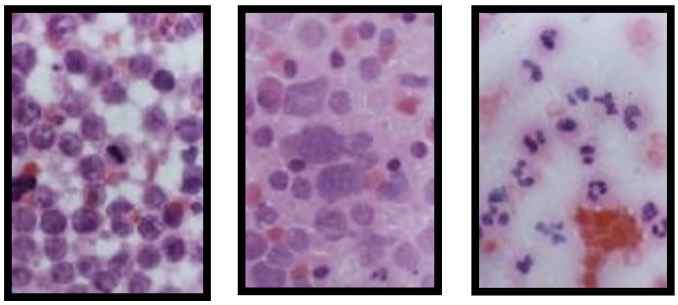
Table 2: Correlation between the results of the biopsy specimens and touch imprint preparations in patients with head and neck mass lesions.

Histological diagnosis	Paraffin section (Biopsy specimens)	Touch imprint cytology (TIC)
Positive for malignancy	15	16
Carcinoma	12	
Lymphoma	3	
Sarcoma	0.0	
Suspicious		2
Negative for malignancy	15	12
Inflammatory lesions	6	
Benign tumor	2	
Non neoplastic polyps	3	
Goiter (colloid and toxic goiter)	4	
Unsatisfactory	0.0	0.0
Total	30	30

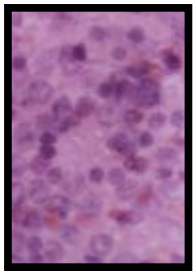
Table 3: Comparison among sensitivity, specificity and predictive values of permanent histologic preparation and touch imprint preparation in the diagnosis of the head and neck lesions.

Aspect		TIC	Biopsy
Total number of cases	[a]	30	30
Total number of malignant lesions	[b]	15	15
Cytology +ve	[c]	16	NA
Suspicious	[d]	2	NA
Negative	[e]	12	NA
Total number without malignancy	[f]	12	15
Cytology +ve	[g]	0	NA
Suspicious	[h]	0.0	NA
Negative	[I]	12	NA
Inadequate cases		0.0	0.0
Sensitivity	[c/c+FN]	88%	NA
Specificity	[I/I+FP]	92%	NA
Positive predictive value	[c/g+c]	100%	NA
Negative predictive value	[I/e+I]	50%	NA

Sensitivity: Ability to detect malignancy, Specificity: Ability to exclude malignancy
FN: False Negative (n=2), FP: False Positive (n=1)
+ve predictive value: +ve cytology report from carcinoma



Non-Hodgkin's Lymphoma Undifferentiated carcinoma Pyogenic inflammation



Pleomorphic adenoma

Figure 1: Representative examples of the touch imprint cytological preparations. Non-Hodgkin's lymphoma, undifferentiated carcinoma, chronic pyogenic inflammation and pleomorphic adenoma of the parotid salivary gland.

Discussion

The aim of the current investigation was to evaluate the value of TIC in the intraoperative examination of ear, nose and throat and head and neck mass lesions. To achieve our goal we simultaneously examined a total of thirty samples both with TIC and permanent histological sections (H&E stain). Our data clearly demonstrate the following findings: 1) TIC is a useful methods for the intraoperative evaluation of the ear, nose and throat and head and neck mass lesions, 2) TIC can provide a sensitive, specific, and time-efficient method to diagnose head and neck mass lesions, and 3) suspicious TIC should not be considered as failure and we should wait for the histology diagnosis.

The high rates of sensitivity and specificity of the TIC was evident in our series. Therefore our data highlight the following advantage for TIC including: 1) it is a relatively simple technique, 2) it can allow the pathologist to render an immediate intraoperative diagnosis, and 3) it is less expensive than the frozen section. Alternatively the disadvantages of the TIC include: 1) it doesn't provide architectural information, 2) it requires considerably experienced pathologist, 3) it can not distinguish between in situ and invasive lesions, and 4) it has the risk of false positive diagnosis.

The cytologic diagnosis "suspicious for cancer" was encountered in two cases. In these two cases, cellularity was lacking, and many bare nuclei and benign cell clusters were present. The cases suspicious for malignancy were negative on further permanent histologic sections. To this end, we propose the necessity of maintaining this diagnostic category for two reasons. First, it allows the cytologist to raise the suspicion of a mass lesion that does not meet all the TIC criteria for malignancy. Second, this diagnostic category helps to keep false positive diagnoses near zero. Nevertheless, this diagnostic term should not be overused. In breast cytology, previous studies indicated that a suspicious category is useful in decision making and should be retained in the analysis of data rather than being combined with positive diagnoses. Moreover, the suspicious TIC needs further correlation with clinical characteristics of the lesions, and then the results of permanent histologic sections should be reviewed.

The false negative cases in our series (two cases) may be inherent in the procedure. Further analysis of these cases, the missed diagnosis could have been averted with reasonable certainty in both of them (pathologist interpretation errors). These false negative diagnoses can be overcome by: 1) more careful screening to detect scant malignant cells especially in bloody smears, and 2) careful clinicopathologic correlation. In frozen sections, many factors can contribute to the false negative rate including the suboptimal preparation of the specimen and sampling errors on the part of the pathologist. To this end, our results indicate that TIC can overcome the deficits of frozen section and proved useful in evaluating head and neck mass lesions (Florell et al., 2001, Hahn et al., 1992, and Gentry 1986).

Recent reports indicated that feasibility of performing further immunohistochemical and molecular analysis on TIC preparations. This diagnostic armamentarium can markedly substantiate the value of TIC in surgical pathology (Salem et al., 2003, Salem et al., 2002, Cserni 2001, Turek et al., 1997, Demetrick, 1996, Ricevuto et al., 1996 Chao et al., 1995, Debongnie et al., 1994, Gentry ,1986 and Helpap and Tschubel, 1977). Whether these techniques are comparably valuable in head and neck mass lesions awaits further investigations. With our results taken into consideration, we strongly believe that the inclusion of TIC in the diagnostic armamentarium for ear, nose and throat and head and neck mass lesions is justified for several reasons. First of all, the reproducibility and the low cost of the procedure. Second, the total absence of false positive makes it not necessary to perform a further frozen section examination. Therefore, the patient can be informed of his or her disease and together with the surgeon can choose the proper treatment. The main disadvantage of TIC is the lack of information about the invasiveness of the tumor, but we believe that this drawback can be overcome by the simultaneous use of permanent histological sections.

In our study, two cases were definitely diagnosed as Non-Hodgkin's lymphoma. These finding concurs with those of other groups (Feinberg et al., 1980 and Koo et al., 1989) and cytoplasmic details of the lymphomatous cells that may be indiscernible indicate that TIC may

reveal nuclear and in even the best tissue sections. Moreover, these findings indicate that TIC may be selectively helpful in contributing to the classification of NHLs (Koo et al., 1989).

To conclude, TIC examination of ear, nose and throat and head and neck mass lesions is fairly accurate in prediction of benign and malignant histologic results. Its correlation with histologic results is sufficient to justify its routine use in immediate counseling and treatment planning.

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Tables

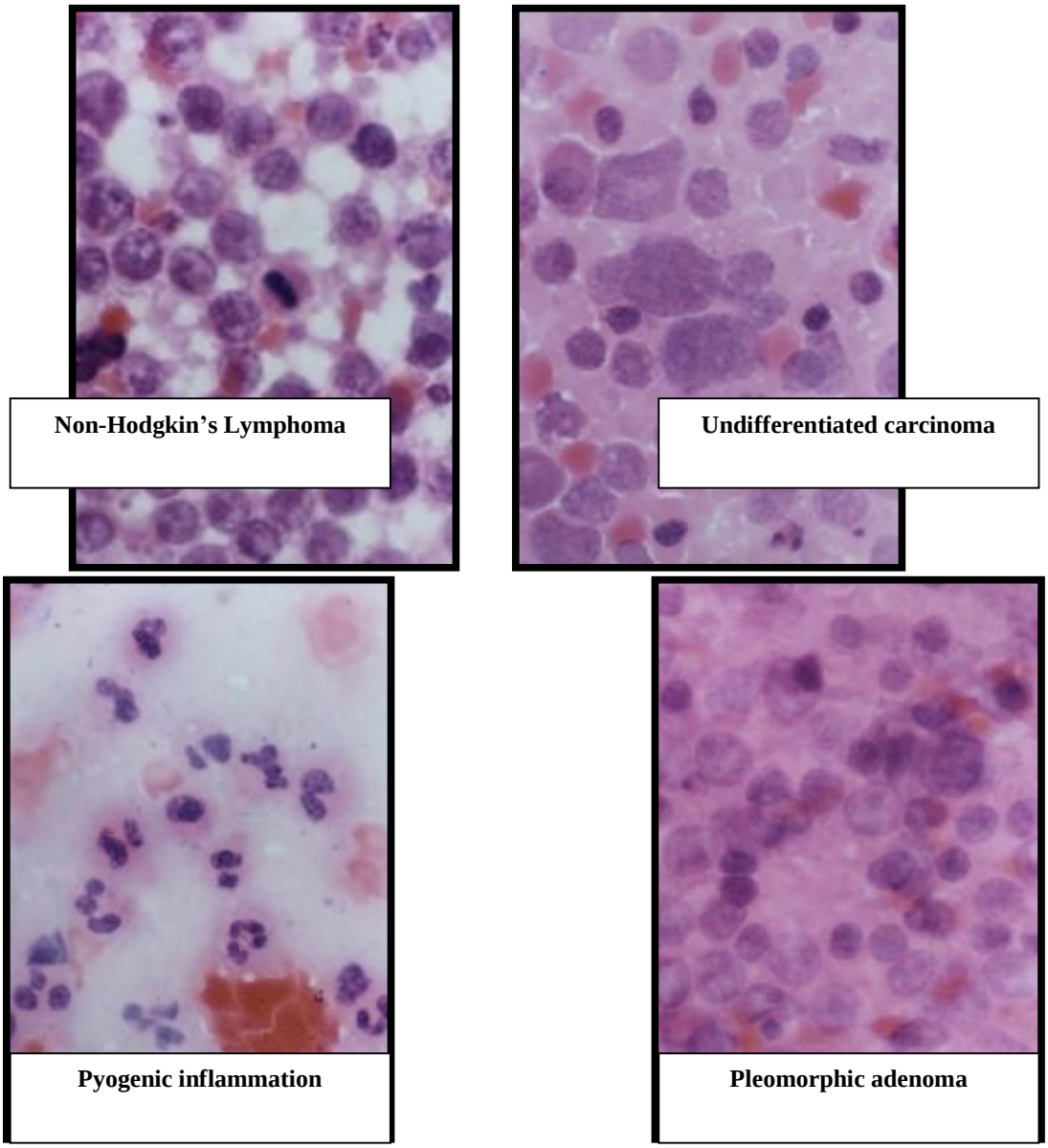
Tables and Figure Legends

Table 1: Clinicopathologic characteristics of the head and neck mass lesions.

M, male; F, female; Sq.C.C., squamous cell carcinoma; NHL, non-Hodgkin's lymphoma and T.B, Tuberculosis.

Table 2: Correlation between the results of the biopsy specimens and touch imprint preparations in patients with head and neck mass lesions.

Figure 1: Representative examples of the touch imprint cytological preparations. Non-Hodgkin's lymphoma, undifferentiated carcinoma, chronic pyogenic inflammation and pleomorphic adenoma of the parotid salivary gland.



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¶ From Pathology*, Ear, Nose and Throat# and Surgery¶ Departments
Assuit University and South Valley University Schools of Medicine, Egypt
Corresponding author
Usama Mohammed Rashad, MD
ENT department, Faculty of medicine
Assiut University, EGYPT
Tel. 093/320322, 0124646101
e-mail: urashad@yahoo.com
Keywords: Touch imprint, head and neck mass lesions
Lecturer, Pathology Dept, Faculty of Medicine, Assiut University
USAMA RASHAD
Lecturer, ENT Dept, Faculty of Medicine, Assiut University
KAMAL ABDULALL ALSHERKAWY¶
Lecturer, Surgery Dept, Faculty of Medicine, South Valley University