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INTRA OPERATIONAL IMPRINT CYTOLOGY AS RAPID CONSULTATION TECHNIQUE IN THYROID LESIONS WITH HISTOPATHOLOGICAL CORRELATION

Pathology

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ABSTRACT

Introduction: Intraoperative imprint cytology thyroid is reliable and cost effective method, provides excellent cellular details. The simplicity, speed and cost effectiveness along with its ability to maximize cell recovery from very small tissue piece makes Touch Imprint Cytology a valuable resource for experimental and diagnostic medicine.
Aims & Objectives: To correlate between imprint cytology & histopathology of thyroid lesions.
Materials & Methods: Intraoperative imprint cytology/scrape preparation of thyroid swelling taken, properly processed, interpreted and course of surgery was done accordingly. Later correlate with histopathology.
Results & Analysis: Results of our study: sensitivity 95.2%, specificity 86.2%, positive predictive value 83.3%, negative predictive value 96.15% and accuracy rate of 90%.
Conclusion: Intra operational diagnosis by imprint/ scrape preparation was rapid as well as cost benefit, can be performed in a tertiary care center where frozen section facility is not available. Thyroid malignancies successfully commented by imprint cytology and scrape preparation. Advantages: rapid intra operational diagnosis of lesion (benign/ malignant) avoid re excisional hazard, better cosmesis, excellent therapeutic action and decreased morbidity. The procedure is safe, rapid, easy, cost effective and very useful procedure in diagnosis of wide spectrum of thyroid diseases.

KEYWORDS

intra operative imprint cytology, scrape preparation, thyroid lesions, carcinoma.

INTRODUCTION:

Imprint cytology is an important tool for diagnosis of various lesions as it is rapid, inexpensive and very useful while awaiting histopathological report where intraoperative diagnosis is urgently needed. The major utility of rapid consultation techniques is to identify and optimize the surgical intervention. Touch imprint cytology is a valuable tool in surgical oncology [1]. As compared to frozen section, it is reliable and cost effective method, and provides excellent cellular details. The simplicity, speed and cost effectiveness along with its ability to maximize cell recovery from very small tissue piece makes touch imprint cytology a valuable resource for virtually every aspect of experimental and diagnostic medicine.

Imprint cytology was first used in hematology as a part of morphological evaluation and immediate comment in bone marrow biopsy material. Intra-operative imprint cytology is an important diagnostic modality in the diagnosis of thyroid lesions. Intra-operative imprint cytology has been receiving increased attention in other areas of pathology, like evaluation of sentinel lymph node biopsy, breast cancer, tumor margins and autopsy. In contrast to frozen section method it can be easily done in under privileged laboratory set up, where facility for frozen section is not available. It has been used in neuropathology for many years and more recently it has been receiving increased attention in other areas of pathology. [2]

In a landmark paper published in 1987, Elvio and Kraemar described the use of various methods for intra operative rapid microscopical diagnosis of tumors [3]. Touch imprint cytology is a valuable tool in surgical oncology. The simplicity, speed and cost effectiveness along with its ability to maximize cell recovery from very small tissue piece makes touch imprint cytology a valuable resource for virtually every aspect of experimental and diagnostic medicine. [4]

In spite of its limitations like inability to differentiate in-situ carcinoma from invasive carcinoma and also inability to provide architectural details, touch imprint cytology has got definite role in intra operative diagnosis that would guide the surgeon's hand.[5]

Imprint cytology can be done by trained personnel with minimal expenditure. [6] The sensitivity and specificity of imprint cytology are comparable to that of frozen sections. [7]

As thyroid nodules rarely prove to be fatal, it is necessary to reduce the number of operation performed. For this purpose, in 2001, Ogawa et al. showed that an accurate diagnosis must be made by imprint cytology. [8]

In 2012, Ali Chehrei et al [9] worked on intra operative touch imprint and crash preparation and compared with frozen section in thyroid nodule.

In another study, Novik et al evaluated patients with thyroid nodules and found the FNA biopsy and/or imprint cytology is very much sensitive and the specific while demonstrating malignancy. [10]

Imprint cytology has excellent preservation of cellular details without any freezing artefact. As there is no loss of tissue, possibility of identifying focal, undetectable neoplastic lesion in large tissue fragments is possible in imprint smear [11,12]During surgery, imprint cytology serves to identify lesions whether it is malignant or not. [13, 14, 15.16]

Issam et al [1], Mutaharra H.A et al. [6] and Belleannee et al. [17] stated that in cases of thyroid malignancies like papillary carcinoma where nuclear features are important for diagnosis, imprint cytology is superior to frozen sections.

The procedure for imprint cytology is a low cost procedure and provides an immediate result with minimal artefacts as described in different literatures reviewed. [6,7,18,19]

Henry MJ et al. [20] shows that application of special stain like Diff-Quik stain may improve the accuracy, sensitivity and speed of imprint cytological diagnosis.

AIMS AND OBJECTIVES:

1. To correlate between imprint cytology & histopathology of thyroid lesions.
2. To evaluate intra operative scrape preparation (ISP) as rapid diagnostic tool.

MATERIALS AND METHOD:

The study was undertaken in the department of pathology of a tertiary hospital for a period of one and half year (2017-2018). It was a hospital based descriptive study of 50 patients admitted in the dept. of ENT with thyroid lesions. The exclusion criteria of study included patients who underwent any form of adjuvant treatment.

For this study, patients with thyroid swelling, attending E.N.T. department and Cytology clinic of department of Pathology were selected. Name, age, sex, address, outdoor registration number and date of the previous FNAC with report were noted.

Patient was prepared for thyroid surgery maintaining strict asepsis at operation theatre.

In the operation theatre, imprint smears were prepared from freshly resected unfixed specimens of thyroid lesions by touching glass slides on the surface, with special focus on suspicious looking area. Before smear preparation blood was removed from the surface of tissue by touching gently on dry gauze piece. Three to four imprint slides were made by gently pressing clean glass slides over the suspected areas of the submitted thyroid tissue. Immediately the slides were submerged in 95% alcohol in a coplin jar.

For scrape preparation, slide was applied gently and tangentially on the freshly resected unfixed specimens of thyroid lesions by touching glass slides on the surface, with special focus on suspicious looking area. After that, gently a clean smear was prepared. Immediately the slides were submerged in 95% alcohol in a coplin jar.

After taking imprint samples (6-7 slides) and scrape preparations, the specimens were handed over to operation theatre in-charge for proper preservation and fixation with formalin and the specimen after proper labeling sent to the department of pathology for histopathological study.

The smears stained by Diff-Quik, MGG, PAP, H&E and modified Giemsa were examined under a microscope thoroughly moving in all directions. The cellular areas of the smear were examined under high power and then under oil immersion.

Analysis was done using statistical software SPSS (Version 20).

RESULTS AND ANALYSIS

The present study of cytological evaluation of thyroid swelling was carried out in the department of ENT and pathology, where a total of 50 patients of both sex and all age groups presenting with thyroid swelling were taken for study. Intraoperative imprint cytology and scrape preparation were carried out in all of them. Histopathological examination done in all cases.

Out of the total 50 cases, satisfactory smears were obtained in 47 cases. Rest 3 cases were labeled as "inadequate for opinion". But, as during surgery a definite conclusion by imprint/ scrape was not made (benign or malignant), the surgical procedure were conducted like malignant lesion to avoid re-excisional hazards.

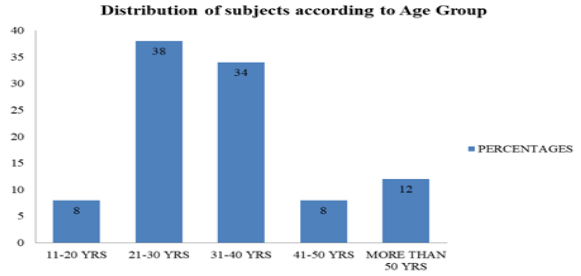


Figure 1: Column Diagram Showing Distribution Of Subjects According To Age Groups (n=50)

The occurrence of thyroid problem and thyroid surgeries were more among 70%(35) females and 30% (15) males in this study population. 62% (31) thyroid swellings were soft in consistency and 38% (19) were firm. 68% (34) thyroid swellings were diffuse and 32% (16) swellings were nodular.12% (6) patients complained of tenderness, 4% (2) had mild tenderness and majority of the patients 84% (42) had no tenderness. Thus, it is vivid that majority of patients had painless thyroid lesions.

Most of the cases 92% (46) had mobile thyroid lesions and only 8% (4) had restricted thyroid swellings.

Table 1: Distribution of Imprint/Scrape Cytological Diagnosis of Thyroid Swelling (n=50)

DIAGNOSIS	NO.OFCASES	PERCENTAGE(%)
ADENOMATOIDGOITRE	11	22
ANAPLASTICCACINOMA	1	2
COLLOIDGOITRE	12	24
FOLLICULARNEOPLASM	6	12
INADEQUATEFOROPINION	3	6
LYMPHOCYTIC THYROIDITIS	1	2
PAPILLARYCARCINOMA OFTHYROID	12	24
MEDULLARY CARCINOMA OFTHYROID	2	4
REACTIVEHYPERPLASIA	2	4
TOTAL	50	100%

In this study, intra operational imprint/ scrape preparation of thyroid lesions helped us to diagnose wide spectrum of thyroid diseases. Among the diseases, 22% (11) was Adenomatoid goitre, Anaplastic carcinoma 2% (1), Colloid goiter 24% (12), Follicular neoplasm 12% (6), Lymphocytic thyroiditis 2% (1), Medullary carcinoma 4% (2), Papillary carcinoma 24% (12) and Reactive hyperplasia was 4% (2). 6% (3) of imprint/ scrape preparation were labelled as "inadequate for opinion". **(Table – 1)**

Table 2: Distribution of Benign (Bethesda II), Malignant (Bethesda VI) Lesions as par Cytological Diagnosis (n=50)

DIAGNOSIS OF THYROID LESIONS	NO.OFCASES	PERCENTAGE(%)
BENIGN	26	52
MALIGNANT	24	48
TOTAL	50	100%

In this study, among the intra operational imprint/ scrape preparation of thyroid lesions, benign lesions were 52% (26) and 48% (24) lesions were malignant. **(Table-2)**

Table 3: Imprint/Scrape cytological diagnosis of various malignancies in thyroid swelling (n=50)

DIAGNOSIS OF THYROID LESIONS	NO. OF CASES	PERCENTAGE (%)
PAPILLARY CARCINOMA OF THYROID	12	24%
ANAPLASTIC CACINOMA	1	2%
FOLLICULAR NEOPLASM	6	12%
MEDULLARY CARCINOMA OF THYROID	2	4%

In this study, intra operational imprint / scrape preparation of thyroid lesions helped us to diagnose various thyroid malignancies. Among the diseases, Anaplastic carcinoma was 2% (1), Follicular neoplasm 12% (6), Medullary carcinoma 4% (2) and Papillary carcinoma was 24% (12). **(Table – 3)**

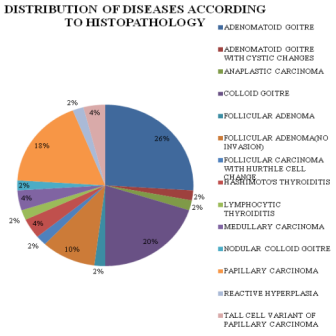


Figure 2: Above pie diagram shows Distribution of Histopathological Diagnosis of Thyroid Swelling (n=50). 26% (13) lesions were diagnosed as Adenomatoid goitre, 2% (1) was Adenomatoid goitre with cystic changes, 2% (1) was Anaplastic carcinoma, Colloid goitre lesions were 20% (10) and Nodular Colloid goitre was 2% (1), Follicular Adenoma was 2% (1), Follicular Adenoma (without invasion) were 10% (5), 2% (1) was Follicular Carcinoma with Hurthle cell change, Hashimoto's Thyroiditis was 4% (2), Lymphocytic Thyroiditis was 2% (1), Medullary Carcinoma lesions was 4% (2), Papillary Carcinoma was 18% (9) and 2 cases (4%) were Tall cell variant of Papillary Carcinoma. Reactive Hyperplasia accounted 2% (1) among the total thyroid lesions.

Table 4: Distribution of Benign, Malignant Lesions as per Histopathology Diagnosis (n=50)

DIAGNOSIS OF THYROID LESIONS	NO. OF CASES	PERCENTAGE (%)
BENIGN	29	58
MALIGNANT	21	42
TOTAL	50	100%

In this study, 58% (29) lesions were benign and 42% (21) lesions were malignant according to histopathology. **(Table-4).**

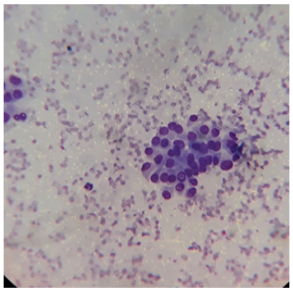
		H/P REPORT for detecting Malignancy		Total
		Positive	Negative	
IMPRINT/SCRAPE for detecting Malignancy	Positive	20(83.3%)(TP)	4(16.7%)(FP)	24
	Negative	1(3.8%)(FN)	25(96.2%)(TN)	26
Total		21	29	50

TP= True Positive, **TN**= True Negative, **FP**= False positive, **FN**= False Negative

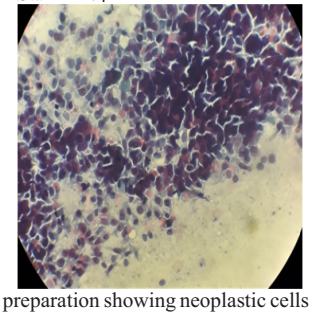
Figure 3: In cases of thyroid lesions, out of 24 positive (malignant) lesions diagnosed by imprint/scrape, 20 cases were true positive and 04 were false positive. Whereas, out of 26 negative cases (non neoplastic and benign), 25 were true negative and 1 was false negative.

Thus, on the basis of observation in the present study, we can find out various parameters for evaluation of intra operative imprint/scrape diagnosis in cases of thyroid lesions, considering the histopathological diagnosis as the gold standard. The sensitivity in this procedure was 95.2% and specificity was 86.2%. The positive predictive value came as 83.3%; negative predictive value was 96.15% with subsequent accuracy rate of 90%.

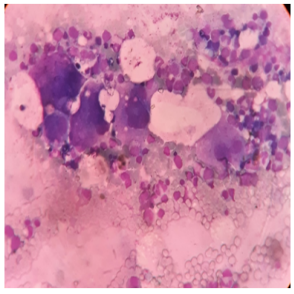
COLOUR PLATES



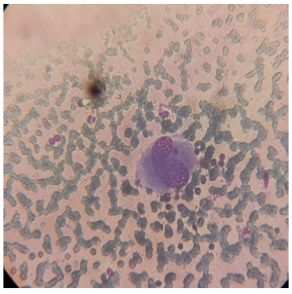
Picture 1: Imprint cytology showing cells are arranged in cohesive clusters with optically clear nucleus (features of papillary carcinoma of thyroid) [Diff-Quik Stain x 40]



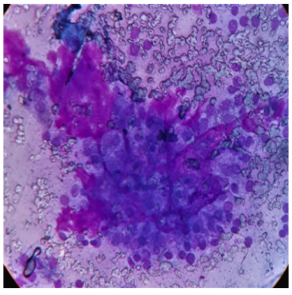
Picture 2: Scrape preparation showing neoplastic cells are arranged in clusters. Individual cells are long, elongated with basally placed nuclei and abundant cytoplasm. Neoplastic cells also have pseudo inclusion and optically clear nucleus. (Tall cell variant papillary carcinoma of thyroid) [PAP Stain x 40].



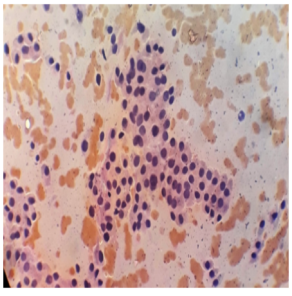
Picture 3: Imprint cytology showing in necrotic background discrete large cells with pleomorphic nuclei, prominent nucleoli, irregular nuclear membrane and abundant eosinophilic cytoplasm. Cytomorphological features of anaplastic carcinoma of thyroid [Diff-Quik Stain x 40].



Picture 4: Scrape cytology showing a neoplastic cell with horse shoe shaped nuclei. Anaplastic carcinoma of thyroid [Modified Giemsa Stain x 100]



Picture 5: Imprint cytology showing in an eosinophilic amyloid background, dissociated cells are round to polygonal in shape having salt and pepper chromatin pattern and reddish granular cytoplasm. (Medullary carcinoma of thyroid) [Diff-Quik Stain x 40]



Picture 6: Imprint cytology showing abundant cellularity in a thin colloidal background (also contains macrophages); cells are arranged in follicular pattern. Cells are monomorphic showing mild large nuclei and fine chromatin and moderate amount of cytoplasm. Features suggestive of follicular adenoma of thyroid [Leishman - Giemsa Stain x 40]

DISCUSSION:

The present study conducted in a tertiary care hospital was undertaken to evaluate importance of imprint cytology and scrape preparation of intra operational thyroid lesions and to use it as a rapid consultation technique to identify and optimize the surgical intervention. The study population included 50 patients.

Thyroid nodule is a common disorder and most of them are benign. As thyroid nodules rarely prove to be fatal, it is necessary to reduce the number of operation performed. For this purpose, an accurate diagnosis must be made.[8] Although thyroid function test, scintigraphy and ultrasonography are routinely used for diagnostic purposes in thyroid nodules, the discrimination between benign and malignant nodule cannot be made exactly with these tests.

At present, preoperative FNAC, intraoperative imprint cytology and frozen section are used for the diagnosis and management of thyroid nodules. Taneri et al (2001) have evaluated the role of imprint cytology as a rapid intraoperative diagnostic procedure as an alternative to frozen section. They have observed promising implications and

suggested this procedure for the small hospitals where frozen section facilities are not available.[18]

Intra operative imprint cytology is rich for cells and thus has been assumed a more suitable cytological examination technique by pathologists, in spite of its limitation for detection of vascular and capsular invasion.[18,21]

Present study was undertaken to demonstrate the value of imprint cytology in the intraoperative diagnosis of thyroid lesions and to compare diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value between imprint cytology and histopathology of resected thyroid specimens. The study included 50 cases presenting with thyroid mass on clinical and radiological examinations. Detailed clinical history of the patients obtained, physical examinations done and routine hematological tests also performed. All subjects having thyroid mass lesions underwent thyroid surgery, where intra operational imprint cytology and scrape preparation were done. The smears were stained with Diff-Quik, H & E, PAP, Leishman – Giemsa and Modified Giemsa stains. All stained smears examined under microscope. In three cases, aspirate was unsatisfactory for evaluation, but, as during surgery a definite conclusion by imprint/ scrape was not made (benign or malignant), the surgical procedures were conducted like malignant lesions to avoid re-excisional hazards. Histopathological confirmation was possible in all 50 cases. Imprint cytology can prevent re excisional or over excisional hazards by quickly prompting the nature of thyroid lesion. Masuda et al. showed that imprint cytology may be useful in prompt intra-operative evaluation.[22]

Sensitivity, Specificity, And Accuracy Of Cytological Imprint Of Different Studies:

STUDIES	SENSITIVITY (%)	SPECIFICITY (%)	ACCURACY (%)
Taneri et al. (2001)[18]	96%	83.3%	97%
Mutaharra et al. (2001)[6]	96%	84%	93.07%
Tworek et al. (1998)[21]	82.55%	94.11%	71%
Dasetal.(1999)[1]	71.15%	85%	100%
Pustaka et al (2011)[23]	90.24%	84.21%	95.45%
Jyanti Et al[24](2015)	91.3%	84.5%	83.63%
Rohit Sharma et al[25](2002)	93.4%	86.8%	84.58%
Singh et al[26](1982)	94.5%	86.4%	84%
Sukumar shaha etal[7](2009)	66.66%	92.59%	81.25%
Presentstudy	95.2%	86.2%	90%

Thus, it is very much clear that our findings of this study are very much similar to previous studies.

The accuracy rate in the present study was 90% which was similar to the accuracy rate of Mutaharra et al. (93.07%), Taneri et al. (97%) and was higher than similar type of studies like Tworek et al. (71%), Jyanti Et al. (83.63%), Rohit Sharma et al. (84.58%), Singh et al. (84%) and Sukumar saha et al. (81.25%)

The sensitivity in present study was 95.2% which was similar to Taneri et al. (96%), Mutaharra et al. (96%) and Singh et al (94.5%).

The specificity of this study was 86.2% which was close to the specificity of Das et al. (85%), Rohit Sharma et al. (86.8%) and Singh et al. (86.4%).

The advantages of Imprint cytology/ Scrape preparation which were noticed during this study were excellent preservation of cellular details without any freezing artifact, no loss of tissue and possibility of identifying focal, macroscopically undetectable neoplastic lesion in large tissue fragments.[11,12] Imprint cytology serves to identify lesions whether it is malignant or not.[13 – 16] The method is effective for determining the parathyroid tissue [27] the sentinel lymph node[28] and the adenomatous goiter. It is also useful in determining the surgical resection margins of neoplastic lesions [29,30].

An accurate diagnosis can be made for metastatic tumors.[31] Imprint cytology is a rapid test and has been applied in the study of various tissues like lymph node, breast, thyroid, etc.[32-36,6,37]

Usually, if a conclusive diagnosis is not reached, the patient is taken up for hemi or subtotal thyroidectomies and later if malignancy is confirmed, a second surgery is done. This is troublesome due to loss of time, cost of re-surgery and increased morbidity. As an outcome of this study, imprint cytology should be the procedure of choice in intra-operative evaluation for single time staging and cost effective management, especially in centers where frozen section facilities are not available.

In thyroid malignancies like papillary carcinoma where nuclear features are important for diagnosis, imprint cytology is superior to frozen sections.[1, 6, 17] There are occasions when FNACs can give false negative results.[38, 39] as in cases of papillary carcinoma in multi nodular goiter or papillary carcinoma associated with Hashimoto's thyroiditis. In imprint cytology multiple smears can be prepared from suspicious areas with the added advantage that the pathologist can see the lesion and make a morphological diagnosis.

The consistent feature to diagnose papillary carcinoma of thyroid by imprint cytology was nuclear character, particularly the nuclear grooving. In a study made by Rupp et al.[40] on nuclear grooving in cytological diagnosis of papillary carcinoma of thyroid, the importance of imprint cytology has been stressed upon.

The procedure for imprint cytology can be done even in underdeveloped infrastructure and deficient trained technician. Analysis of an individual cell is performed by imprint cytology. It provides an immediate result with minimal artifacts, it is cheaper and so it is most commonly used. A precise diagnosis is received through this technique.[6, 7, 18, 19] Application of special stain like Diff-Quik stain may improve the accuracy, sensitivity and speed of imprint cytological diagnosis.[41]

Limitation:

The limitations of this study included absence of pre-operative feedback regarding type of the lesion (benign or malignant) cannot be given as it is an intra-operative procedure. At times, it is hard to diagnose non neoplastic lesions due to pauci-cellular smear in imprint smear and scrape preparation. As the pattern is not obvious in imprint smear, so, there is a great limitation in determining the behavior of capsular/vascular invasion.

CONCLUSION:

Intra operational diagnosis by imprint/ scrape preparation was rapid (by Diff-Quik stain) as well as cost benefit, particularly in a tertiary care centre, where frozen section facility is not available. In this study, thyroid malignancies are almost successfully commented by imprint cytology and scrape preparation. Non neoplastic lesions are very difficult to comment in imprint/scrape as the cellular yield is much less, though abundance of colloid may give some hints like cytology favoring benign nature. Statistical analysis had shown significant positive outcome in imprint smear as well as in scrape preparation with excellent sensitivity (95.2%) and specificity (86.2%) with high positive predictive value (83.3%) & diagnostic accuracy (90%).The advantages of intra operational imprint cytology are rapid intra operational diagnosis of lesion (benign/ malignant), avoid re excisional hazard and help the surgical decision regarding block dissection of lymph nodes, better cosmesis, excellent therapeutic action and decreased morbidity. Finally, procedure is a safe, rapid, easy, cost effective and very useful procedure in diagnosis of wide spectrum of thyroid diseases and helps better therapeutic outcome.

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