e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(9); 1402-1407

Original Research Article

Study of Fine Needle Aspiration Cytology and Immunohistochemistry in the Diagnosis of Cervical Lymphadenopathy

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Received: 25-06-2023 / Revised: 28-07-2023 / Accepted: 30-08-2023

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Conflict of interest: Nil

Abstract:

Background: Aspiration cytology and histopathological study with the help of FNAC have been used for the diagnosis purpose but sensitivity and specificity still remain away for final conclusion. Immunohistochemistry asses the tumour markers of infiltrating lymph nodes and helps to determine independent marker associated with better diagnostic accuracy.

Aim: To study the diagnostic accuracy of FNAC and Immunohistochemistry for the cervical Lymphadenopathy.

Methods: A Prospective study of 106 patients attended surgery OPD with prior information and consent was done in the period from 2016 to 2018 after approval of IEC. FNAC was done in 102 cases and then were referred for open biopsy. Sides and different levels were delineated with the help of land mark stiches placed just after the removal of the specimen. Specimens (28 cases out of 102) with inconclusive diagnosis or difficult diagnosis by histopathological study were sent for immunohistochemistry with cytokeratin test. Then the reports of FNAC, HPE and immunohistochemistry reports were compared and analysed for their diagnostic accuracy.

Results: There is statistically significance when the tests were compared for age of patients, level of lymph node involved and pathological diagnosis like reactive hyperplasia, TB lymphadenitis and malignant conditions like SCC and adenocarcinoma. FNAC was more diagnostic for benign diseases than malignancies and more likelihood+ for primary lymph node malignancies than metastatic disease. The ratio of non- neoplastic to neoplastic was 6.21:1. Requirement of immunohistochemistry for the final conclusion for the pathological diagnosis was 25% but the diagnostic accuracy was 90.32% and more likelihood+ for diagnosis by immunohistochemistry for metastatic lymph node disease.

Conclusion: Histopathological examination of neck dissection specimens is highly sensitive and specific for detection of metastatic deposits with high diagnostic odds ratio for lymphadenopathy. Micro metastases or sub pathological metastases are undetected by H&E staining but detected by immunohistochemistry or molecular analysis. The cytokeratin immunohistochemistry has come in a very handy and useful tool by unearthing the metastasis in those lesions which were decided to be non-malignant by histopathological examination. Immunohistochemistry is having with highest sensitivity, specificity and diagnostic odds ratio for micro metastases detection and molecular markers.

Keywords: Fine Needle Aspiration Cytology, Immunohistochemistry, Diagnostic Accuracy, Cervical Lymphadenopathy.

Introduction

Lymphatic tissue confined in the lymph nodes is responsible for immunological response along with other functions. Lymph nodes are structured with both afferent and efferent lymphatics distributed throughout the body responsible for drainage lymphatic fluid. Lymph nodes distributed in the head and neck regions drain dominantly from head and neck area. Drainage lymph nodes can be involved with inflammatory cells, malignant cells invasion and hyperplasia of lymphatic tissue. Aspiration cytology and histopathological with the help of FNAC have been used for the diagnosis purpose but sensitivity and specificity still remain away for final conclusion. Employment of Immunohistochemistry for diagnosis of suspicion malignancy can improve the diagnosis and better

management [1]. Cervical lymph node involvement and diagnosis of melanoma remains inconclusive by USG guided biopsy. Cancer cell dissemination to the sentinel node is a quantitative risk factor for melanoma death.

Quantitative measurement of sentinel lymph node for melanoma with the help of immunohistochemistry may be useful clinically [2]. Any diagnostic method for detection of occult metastases in sentinel lymph node could change post-operative therapeutic management. Use of immunohistochemistry for diagnosis of sentinel lymph node metastases in patients with breast cancer enables detection of occult metastases [3]. Sentinel nodes deserve more attention including step sections and immunohistochemistry which increases the percentage of metastases found in lymph node [4]. Molecular techniques such as DNA sequencing and immunohistochemistry have identified molecular biomarkers associated with Extracapsular spread from lymph nodes can impact prognosis[5]. TREM2 expression was analysed in the primary tumours and corresponding lymph node metastases of oral squamous cell carcinoma patients via immunohistochemistry on tissue microarrays [6]. Immunohistochemistry asses the tumour markers of infiltrating lymph nodes and helps to determine independent marker associated for better survival [7]. Squamous cell carcinoma of head and neck is one of the most common cancers with a global incidence of 888,000 new cases per year [8].

The presence of cervical lymph node metastasis in head and neck cancers is known to be the single most adverse prognostic factors[9]. The no of lymph nodes, the levels in the neck involved, the size of the metastases and the presence of macroscopic or microscopic extracapsular spread affect the final outcome of neck metastases in patients with HNSCC [10]. The frequency of metastatic lymph nodes at various neck levels as level 1- 10%, level 2- 75%, level 3- 51.7%, level 4- 13.5%, level 5- 1%[11]. Cervical lymph node metastases from unknown primary sites account for approximately 3% to 9% of all head and neck malignant lesions[12].

The presence of lymph node metastases could be detected by clinically, radiologically and by ultrasound guided fine needle aspiration cytology [13]. None of the imaging modalities have been proved to be sensitivity and specificity 100% for diagnosis cervical lymph node metastases [14]. Histopathological examination of neck dissection specimens has highly sensitivity and specificity for detection of metastases [15]. Immunohistochemistry is the most common method used to detect cytokeratin in metastatic cells in lymph nodes [16].

Aim

To study the diagnostic accuracy of FNAC and Immunohistochemistry for the cervical Lymphadenopathy.

Materials and Methods

A Prospective study of 106 patients attended surgery OPD at SCB Medical College and hospital, Cuttack with prior information and consent was done in the period from 2016 to 2018 after approval of IEC. The patients were referred to pathology department for lymph node biopsy for confirmation of diagnosis. Informed consent was taken from the patients who were undergone surgery for lymph node biopsy and for the study.

The cases with incomplete data were excluded. The classification of the America otolaryngology, Head and neck surgery was used to determine the level of nodal involvement. FNAC was done in 102 cases and then were referred for open biopsy. Sides and different levels were delineated with the help of land mark stiches placed just after the removal of the specimen. Each level of nodes was sent in different container and the specimen was sent for HPE. Specimens (28 cases out of 102) with inconclusive diagnosis or difficult diagnosis by histopathological study were sent for immunohistochemistry with cytokeratin test. Then the reports of FNAC, HPE and immunohistochemistry reports were compared and analysed.

Technique of Histopathology

Tissue procurement and preparation starts with fixation and fixative are acetic acid formaldehyde, ethanol, glutaraldehyde, methanol and picric acid. Fixative solutions are 10% neutral buffered formalin, 4% paraformaldehyde. Methods of fixation are immersion which is commonly used or embalming.

The tissue is embedded in a solid medium firm enough to support the tissue and give it sufficient rigidity to enable thin sections to be cut (3-10 micrometre). Specimens were dehydrated in a graded ethanol for 30 minutes or 90 minutes for minimum consistent with 1 mm block or 5 mm block. Clearing is replacement of the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium. clearing agents are Xylene, Toluene, chloroform, Benzene or petrol. Embedding is the process by which tissues are surrounded by a medium such as agar, gelatin or wax which when solidified will provide sufficient external support during sectioning and hardened by replacing water with paraffin. The tissue was cut in the microtome at thickness varying from 2 to 25 micrometres thick. The tissue mounted on microscopic slide, stained, and examined on light microscope.

Technique of Immunohistochemistry

Paraffin sections were deparaffinized in Xylene, hydrated with 95% ethanol and rinsed in distilled water. Antigenic determinants masked by formalin - fixation and paraffin -embedding often may be exposed by epitope unmasking enzyme digestion or saponin etc. Sections were rinsed in PBS -Tween 20 for 2*2 min. Then sections were incubated with normal serum block, for 30 minutes.to block nonspecific binding of immunoglobulin. Sections were incubated with primary antibody (cytokeratin) at appropriate dilution in primary antibody dilution buffer for 1 hr. at room temperature or overnight at 4 degree centigrade. Rinsed in PBS -Tween 20 then sections were incubated in peroxidase blocking solution for 10 minutes at room temperature. Rinsed in PBS -Tween 20. Sections were incubated with biotinylated secondary antibody at appropriate dilution in PBS for 30 minutes at room temperature. Rinsed in PBS -Tween 20 for 3*2 minutes. Sections were incubated in streptavidin HRP in PBS for v30 minutes at room temperature. Rinsed in TBS for 3*2 min. Sections were incubated in DAB solution for 1-3 minutes. Rinsed in PBS -Tween 20 2*2min. Counterstained if desire. Rinsed in distilled water dehydrated through 95% ethyl alcohol and cleared in Xylene for 2*5 min. Coverslip applied with mounting medium. The new preparation of cytokeratin stain was reviewed by same pathologist.

Statistical Analysis

Clinical demographic characteristics related to diagnostic procedures with the conclusions were compared between the 72 patients under gone FNAC / HPE and the other 28 cases remained inconclusive after HPE and done immunohistochemistry with cytokeratin for conclusion. The means of continuous variables were compared by paired t test and categorical variables were compared by McNemar's chi-square test. Diagnostic accuracy in the form of PPV, NPV, Sensitivity, specificity, Likelihood+\-, diagnostic accuracy and diagnostic odds ratio of the tests for different pathologies were calculated and compared. The statistical analysis was done with the help of spss22.

Observations and Results

Table 1: Clinical characteristics and Correlation for diagnostic accuracy of cervical lymph adenopathy

Clinical and demographic characteristics	FNAC and histopathological Study (102)	Immunohistochemistry with cytokeratin (28)	P-Value (significant p=<0.05)
Age(Mean age)	37.2 years	31.4 YEARS	0.03
Sex{M/F)	1.5:1	2.2:1	0.39
Level-1 group lymph nodes	38 (52%)	8%	0.01
Level-2	13(17%)	28%	0.09
Level-3	09(12.5%)	24%	0.06
Level-4	14(18%)	22%	0.23
Level-5	16(25%)	8%	0.02
Level-6	07(11%)	3%	0.09
Level-7	03(5%)	7%	0.33
Reactive hyperplasia	26%	3%	0.004
TB lymphadenitis	16%	3.51%	0.03
Other benign conditions	2%	3.5%	0.37
SCC	36%	82%	0.008
Adenocarcinoma	20%	9%	0.08
Hodgkins Lymphoma	4%	3%	0.40
Non-Hodgkins Lymphoma	1.5%	4%	0.20
Fever	18%	38%	0.01
Cough	18%	28%	0.12
Loss of appetite	12%	25%	0.04
dysphagia	2%	22%	0.005

Table 2: Diagnostic accuracy tests for FNAC

	(TP)(a)	FP(b)	FN©	TN(d)	PPV	NPV	Sensitivity	Specificity	Likelihood+	Likelihood-	Disease prevalence	Diagnostic accuracy	DOR
Metastatic	56	17	25	21	76.71%	45.65%	69.13%	55.26%	1.54	0.55	68.06%	64.70%	2.76
Benign	44	16	02	56	73.33%	96.55%	95.65%	77.77%	4.30	0.05	38.98%	84.74%	77.0
Primary Malignancy	06	02	02	94	75%	97.91%	75%	97.91%	35.88	25.53	7.6%	96.15%	1.41

Table 3: Diagnostic accuracy tests for of immunohistochemistry

	(TP)(a)	FP(b)	FN©	TN(d)	PPV	NPV	Sensitivity	Specificity	Likelihood+	Likelihood-	Disease prevalence	Diagnostic accuracy	DOR
Metastatic	25	01	02	03	96.15%	60%	92.59%	75%	3.70	0.09	87.09%	90.32%	41.11
Benign	02	00	00	26	100%	100%	100%	100%	1.01	0.99	7.14%	100%	1.02
Primary Malignancy	02	00	00	25	100%	100%	100%	100%	1.01	0.99	7.4%	100%	1.02

In our study of 100 cases of cervical lymph lymphadenopathy, 61 cases were non-neoplastic lesions and 39 cases were neoplastic lesions and the ratio was 1.56:1. Majority patients referred for FNAC were 44% in the age group of 30-40 years. Mean age of the patients done FNAC were 37.2 years and that of immunohistochemistry were 31.4 years. Majority patients referred for FNAC were 44% in the age group of 30-40 years. Majority of patients were male (64%) and 36% were female with M:F ratio 1.4:1 There is statistically significance when the tests were compared for age of patients, level of lymph node involved and pathological diagnosis like reactive hyperplasia, TB lymphadenitis and malignant conditions like SCC, Adenocarcinoma (Tab: 1). FNAC is more diagnostic for benign diseases than malignancies and more likelihood+ for primary lymph node malignancies than metastatic (Table-2). The ratio of non-neoplastic to neoplastic was 6.21:1. 56% cases were metastatic squamous cell carcinoma, papillary carcinoma of thyroid or adenocarcinoma as diagnosed by FNAC whereas 24.9% case were undetected for metastatic lymph nodes were confirmed by immunohistochemistry with 100% sensitivity and specificity. 28% cases were proved to be benign by FNAC with sensitivity 98.83% and specificity 100% while 1 case i.e., 0.9% was confirmed to be TB lymphadenitis with sensitivity and specificity 100% and 100% respectively. 6% case was detected as primary malignancy for HL and NHL whereas 2 cases were undetected by FNAC with sensitivity 93.3% and confirmed after immunohistochemistry with sensitivity and specificity 100%. Requirement of immunohistochemistry for the final conclusion for the pathological diagnosis was 25% but the accuracy was 100%. Diagnostic accuracy 90.32% and more likelihood+ for diagnosis by immunohistochemistry for metastatic lymph node disease (Table-3) Pathological variants require immunohistochemistry for final conclusion was specific. Most of them were squamous cell cancer metastasis from distant primary.

Discussion

All cases of lymph nodes lesions presenting with superficial cervical lymphadenopathy where FNAC needs to be done and those cases undergoing subsequent biopsy and immunohistochemistry were studied. In our study of 100 cases of cervical lymph lymphadenopathy, 61 cases were non-neoplastic lesions and 39 cases were neoplastic lesions and the ratio was 1.56:1. Majority patients referred for FNAC were 44% in the age group of 30-40 years. Majority of patients were male (64%) and 36% were female with M: F ratio1. 4:1. the ratio of non-neoplastic to neoplastic was 6.21:1. Most common site of cervical lymphadenopathy was posterior triangle i.e., level 5 and level 6 which were inconclusive by FNAC and histopathology was referred for immunohistochemistry. Out of 24 cases lymphomas 19 cases were non-Hodgkin lymphoma and 5 cases were found to be Hodgkins lymphoma. The commonest site of primary in cases of metastatic secondaries was oral cavity followed by thyroid and larynx, Lungs and GI tract. In case of metastatic secondary's, FNAC had sensitivity, specificity, Twelve cases of metastatic deposit was missed out both in FNAC and histopathological examination which was later confirmed by cytokeratin immune histochemistry. Sensitivity, specificity and diagnostic odds ratio for immunohistochemistry were 92%, 75% and 41.11 for metastatic lymphadenopathy respectively. Other sixteen cases were confirmed to be benign by immunohistochemistry were reactive lymphadenitis and TB lymphadenitis. Similar studies as per Kaur A et al FNAC was more useful and sensitive in diagnosing the metastatic cervical lymph nodes and the accuracy can be further improved by the use of IHC on the cell blocks [17]. In 72 primary ADCs, TTF-1, Napsin A and CK7 showed a sensitivity and specificity of 84.5%/96.4%, 92.0%/100%, and 93.8%/50.0% as studied by Gurda GT et al[18]. Twenty-five percent (25%) of the reactive hyperplasia's on FNAC ($p < 0.0001$), 33.3% of inadequate FNAC ($p = 0.003$) and 75% of atypical cells in FNAC turned to be malignant on lymph node biopsy with a discordance rate of 20.3% as studied by Newton MV et al [19]. Flow cytometry and immunocytochemistry were used in 67 cases and the FNAC diagnosis had a sensitivity of 95.5% and a PPV of 96.8% in this group as studied by Hay A et al [20]. For HE, the sensitivity, specificity, NPV and accuracy were 77, 100, 94, and 95 %, respectively. With subsequent analysis with SSS/IHC, these values increased to 92, 100, 98 and 98 %, respectively as studied by Chone CT et al [21].

Conclusion

Histological analysis is central to the assessment of these lymphadenopathies when clinical or cytological criteria alone are unable to establish a precise diagnosis and especially in high suspicious for malignancy cases. Histopathological examination of neck dissection specimens is highly sensitive and specific for detection of metastatic deposits with high diagnostic odds ratio for lymphadenopathy. Micro metastases or sub pathological metastases are undetected by H&E staining but detected by immunohistochemistry or molecular analysis.

The cytokeratin immunohistochemistry has come in a very handy and useful tool by unearthing the metastasis in those lesions which were decided to be non-malignant by histopathological examination. Immunohistochemistry is having with highest sensitivity, specificity and diagnostic odds ratio for micro metastases detection and molecular markers. It may be recommended for routine diagnostic use in patient with negative for lymph node metastases on routine haematoxylin-eosin stain before deciding a lesion as non -malignant.

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