Study of Cytokeratin AE1/AE3 Reactivity in Squamous cell carcinoma in aspiration cytology

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Abstract

Over: expression of Cytokeratin CK has been reported in malignant cells in aspiration cytology. Immunocyto-chemical detection of cytokeratin is an easier technique than molecular detection in malignant cells. Hence we investigated the presence of this protein in the most common malignant cells.

Material and methods: We performed immunocytochemical ICC detection of CK in aspirate cytology of malignant cells with or without inflammatory and benign cells and correlated with varied patterns of CK positivity with respect to cytological diagnosis.

Results: In the present study 40% cases of malignant cells were found positive for CK of which the most common pattern observed was diffuse cytoplasmic staining. Among the cases with SCC 66.6% were positive for over-expression of CK wherein diffuse pattern was observed.

Conclusion: In the present study, significant number of SCC in aspirate cytology cases observed over-expression of CK. However SCC detection in aspiration cytology is a morphology based technique.

Keywords: Immunocytochemistry, cytokeratin, aspirate, Squamous cell Carcinoma.

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Introduction

In the United States, carcinoma is a second only to cardiovascular disease as the leading causes of death. In the United Kingdom it is the leading cause of death. In India the total cancer causes are likely to go up from 936,908 cases in the year 2010 to 1,044,650 cases in the year 2020(1).

The etiology of carcinoma has been considered to be multifactorial and dietary habits and life style factors play a major role. Several epidemiological data suggest a strong association like cigarette smoking, tobacco and alcohol consumption further, there between chewing betel quid with and without tobacco and oral squamous cell carcinoma [2-5].

CK is an intermediate filament and essential cytoskeleton component involved in maintenance of cell morphology, observed mainly in epithelial cell [6]. Cytokeratin AE1/ AE3 is a mixture of two different clones of anti- cytokeratins monoclonal antibody. It is detected all type of molecular (low molecular as well as high molecular weight keratins) due to this combination some pathologist cytokeratin AE1/AE3 called as pancytokeratins. By combination of these two reagent, a single reagent with a broad spectrum of reactivity against both high and low molecular weight cytokeratins is obtained[7].

In dysplasia and carcinoma in situ, atypical cells showed reaction patterns in which loss or increased AE1 expression was present suggesting that AE1 could be used as a biomarker for identifying early aberration in esophageal epithelial carcinoma. The relationship between the morphologic characteristics and in-situ hybridization for CK-mRNAs and demonstrated that changes in CK expression occur with differences in malignant potential in the esophageal squamous epithelium [8].

Fine Needle Aspirate Cytology FNAC is a technique used to obtain material from organs that do not shed cells spontaneously. It is valuable in the diagnosis of the lesions of breast, thyroid, lymph nodes, liver, lungs, skin, soft tissues and bones. It is now a widely accepted diagnostic procedure, which has largely replaced open biopsy [9].

Apart from FNAC smears, ICC can be done on smears prepared from centrifuged cell deposits obtained from body fluids and cells grown in culture[10].

Materials and methods

On the basis of clinical features and cytological confirmation n=45 patients are included in present study in which n=12 inflammatory lesion n=13 benign and n=20 malignant lesions are included. This study

was conducted in Ruxmaniben Deepchand Gardi Medical College, Ujjain (m.p.) between 2011 to 2012. All cases included in the present study were clinically suspicious for malignancy. All HIV positive patients and patients below 10 year of age were excluded from the study.

Fine needle aspiration procedure was carried out under strict aseptic precautions in the department of pathology after taking informed consent of patient. After the sample collection, routine cytological examination and Staining of slides for cytology was done by using Leishman's stain and Giemsa stain.(Bio-Lab diagnostics).

ICC was performed using Polymeric (Envision TM Flex mini kit Dako K8023) technique on cell smear preparation, which was prepared as earlier. These were air dried and kept at 2-8^{o} C till further used. Envision system- It is based on dextran polymer technology which permit binding of a large number of enzyme molecules to a secondary antibody via dextran background. Antibodies used in the study were optimally pre-diluted and were ready to use. The staining kit was provided by Dako (Code number 8023).Cytokeratin AE1/AE3 monoclonal (Mouse) Diaminobenzidine Dako(Code number IS053) was used. The distribution of CK AE1/AE3 positivity was scored as negative (<5% cell positive), sporadic (<10% cell positive), focal (<25% cell positive), and diffuse (>25% cell positive).

Ethical approval: The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2008, and had the approval of the departmental ethics Committee.

Results

The present study is based on cytological evaluation and immunocytochemical study of 45 cases, where patients presented to out patient department for Fine needle aspiration of Lumps and swellings.

1. Demographic characteristic of cases –

Patients were having the age variation from 17 to 70 years with the mean age of 45.8 years. The maximum numbers of cases (48.8%) were present in the older age groups (5th and 6th decade). We found more number (55%) of malignancies in fine needle aspiration cytology were present in older age group. In our study, 53.3% of total cases were males with male to female ratio 1.3:1.0.

Table 1: Demographic distribution of case in aspirates

Aspirates	Inflam-matory	Benign	Mali-gnant	Total
Age groups in (Years)				
0-30	06	03	03	12
31-60	05	08	14	26
>60	01	02	03	06
Gender				
Male	08	07	11	26
Female	04	06	09	19
Total	12	13	20	45

2. Clinical causes in different effusions -

As lymph nodes are sites for metastatic deposits high rate of malignancies were seen in lymph node aspirates, seven lymph nodes cytology were diagnosed as malignant. Majority of the aspirates from thyroid (n=3), breast (n=3), ovary (n=2) and liver mass (n=2) also showed malignant change. There were few cases in which aspirates from organs like lung, soft tissues and mediastinum were found positive for malignant cells.

Table 2: Distribution of case according site of aspiration

Site	Inflam-matory	Benign	Malig-nant	Total
Lymph node	09	02	07	18
Salivary gland	02	03	00	05
Thyroid	01	02	03	06
Lung	00	00	01	01
Breast	00	00	03	03
Liver mass	00	00	02	02
Ovary	00	01	02	03
Miscellaneous	00	05	02	07
Total	12	13	20	45

3. Routine examination of aspirates

Gross appearance

Majority (n=40) of the aspirate were hemorrhagic and even among inflammatory cases (n=12) seven cases had blood mixed aspirate.

Microscopic finding

Majority of inflammatory and benign cases had variable cellularity, however few smears were Paucicellular while 11 out of 20 malignant aspirates showed high cellularity. Out of 12 inflammatory aspirates eight aspirates showed lymphocyte as the predominant cell with macrophages and epithelioid cells. In the malignant lesions maximum cases showed high cellularity with varying pattern of lesion as diffusely scattered sheet of cells, acinar or ball like clusters of cells. Smears showed cytological criteria of

malignancy with variable amount of cytoplasm in cells, enlarged hyperchromatic & pleomorphic nucleus increased nucleo-cytoplasmic ratio and cells showed mitotic figures.

4. Immunocytochemistry of aspirates

All the smears were subjected to immunocytochemical staining using antibody against cytokeratin and benign / inflammatory lesion were subjected to ICC as negative controls.

Table 3: Distribution of case according cytological typing with CK positivity

Cytology	CK		Total
	Positive	Negative	
1. Infammatory	00	12	12
2.Benign	00	13	13
3. Malignant			
a. Adenocarcinoma	00	04	04
b. Squamous cell carcinoma	08 (66.6%)	04 (33.3%)	12
c. Other	0	04	04
Total	08 (17.7%)	37 (82.2%)	45

Cytokeratin - Cytokeratin positivity was found (**Figure-1**) in all epithelial cell tumors including squamous cell carcinoma and mesothelioma. It does not give positive immunostaining in the cases of adenocarcinoma. Unequivocal cytoplasmic staining observed in > 20-30 percentage tumor cells were consider positive. Out of the 20 cases of malignant cells, eight (40%) cases were showing positivity.

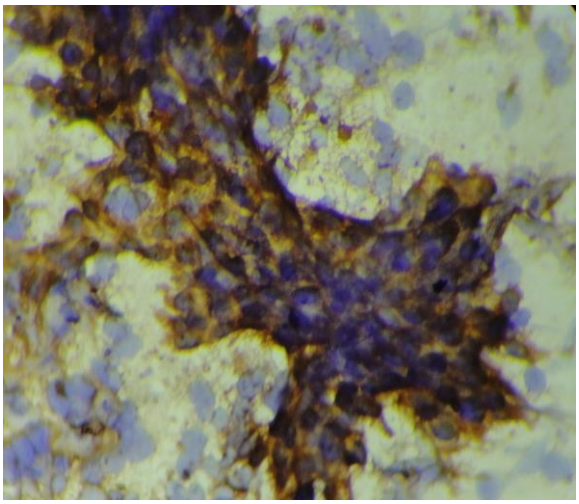

Fig. 1: Cytokeratin AE1/ AE3 Positive in Metastatic Squamous cell carcinoma (40x)
FNA done by supra clavicular lymph node.

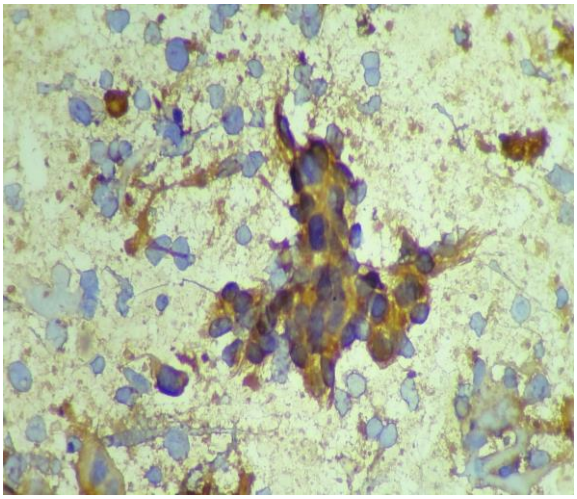

Fig. 2: Cytokeratin AE1/ AE3 Positive in Squamous cell carcinoma (40x)

Discussion

The purpose of this study was to compare the immunocytochemical reactivity patterns of antibodies with cells in malignant and benign aspirates (posing as negative control) and to assess their potential value in routine diagnostic cytology. The use of the immunocytochemical procedures has been advocated by several workers as helpful in often difficult differential diagnosis. It has been shown that immunocytochemistry is an important diagnostic tool for differential diagnosis of various tumors. So far, a number of antibodies have been applied to serous aspirates with varying degree of efficacy.

On fine needle aspiration cytology, gross finding and cellularity were not criteria to differentiate between benign and malignant cases however; these can only be differentiated by the features of cellular pleomorphism, nuclear atypia, prominent nucleoli and cell type. In present study, out of 45 aspirates, 20 aspirates were diagnosed as malignant with cellular features of cellular pleomorphism and anaplasia. We found that out of nine inflammatory aspirates five were showing features of tuberculosis as histiocytes, necrotic background and groups and scattered slipper shaped epitheloid cells. Occassional smear also showed a giant cell probably as Langhan's giant cell. The high prevalence of tuberculosis in India explained the most common reason of lymphadenopathy especially at superficial and peripheral sites. In tuberculosis prevalent countries tuberculosis is the most common cause of cervical lymphadenopathy [11]. Almost a quarter of the aspirates from the lymph nodes showed reactive changes. Similarly, in salivary glands 40% of the cases were inflammatory in origin. About 15% of thyroid lesions showed inflammatory changes. Among these head and neck lesions only a minority showed acute or chronic inflammation.

We found that maximum number of aspirates showing malignant cells were from lymph nodes. This asserts lymph node as the primary site of the metastasis

from the head and neck primaries apart from being a source of lymphomas. Among thyroid malignancies female preponderance clearly emerged as more than 60%, malignant lesions were found in the female. All the aspirates from breast were showing malignant lesions. The lesions of hepatic and ovarian origin turned out to be malignant in majority of cases and confirmed by immunocytochemical assessment.

Immunocytochemical techniques have now become widely used in cytopathology for the demonstration of a large number of various antigens (e.g. p53, CEA, EMA, LeuM1, B72.3, Lectin, cytokeratin, vimentin etc) in effusion and aspirate as an aid in differentiating malignant cells from benign cells. Nevertheless, for a particular carcinoma, the use of a single marker appears insufficient because it is not always expressed and in addition, no tumor marker has a spectrum wide enough to detect all types of malignancies. In the present study, we studied Cytokeratin.

The detection of cytokeratin intermediate filament is widely used to identify tumors of epithelial origin. In present study cytokeratin positivity is found in 08/12 (66.66%) squamous cell carcinoma from aspirates. Among these 08 cases, one case on cytological examination was suspected as mesothelioma and that turned out as squamous cell carcinoma by showing positivity for CK. Immunostaining was negative for CK in all the cases of adenocarcinoma. As per Azevedo et al squamous cell carcinoma of salivary gland showed immunopositivity for CK 6,7,8,14,19. They showed CK positivity of squamous cell with varying result from 54% for CK 19, to 80% for CK7 [12]. In present study, we found that 66% cases were positive for CK AE1/AE3 (cocktail).

In series of cases, CK7 and CK19 positive was detected in adenocarcinoma of gastro-esophageal junction, CK7 and CK19 were found positive in 90% of the cases [13]. This can be attributed to the presence of squamous cells near the gastro-esophageal junction. In the present study the above mention sites was not undertaken although metastatic deposits of squamous cell ca in lymph node comprised most of the cases. Among this group CK AE1/AE3 was found to be positive in 66%. This shows that the high rate of positivity achieved in the above-mentioned study was more due to cells of squamous differentiations rather than adenocarcinoma.

According to Kaufmann et al a study conducted on poorly differentiated squamous cell carcinoma, 84% case were positive for CK 5/6 where as in the present study CK AE1/AE3 was found positive in the 66% of cases [14]. In the same study 93% of squamous cell carcinoma of the lung were positive for CK 5/6 were as in our present study 100% positive in aspirate and 66% in effusions [14].

Sandra j shin et al undertaking research on fine needle aspirate biopsy of metastatic deposits it was found that, CK AE1/AE3 was positive of 14 out of 16 cases [15]. We found that all the cases of metastatic squamous cell carcinoma stained positive for CK AE1/AE3. Strong staining for keratin is evident in all squamous cell carcinoma (Gusterson, Michell, Warburton and Sloane 1982; Said, Nash, Tepper and Banks-chlegel 1983; Schlegel, Schlegel, McLeod and Pinkus 1980). This reaction is particularly helpful in identifying squamous differentiation in poorly differentiated areas. Thus, we hoped that the marker would predict the histological type and even the origin of the tumour. We had four lymph node biopsy showing squamous cell carcinoma and the aspirate from the same lymph gland showed CK positive on immunocytochmical staining thus confirming the origin of the tumor cell. One of this case showed a poorly differentiated form of squamous cell carcinoma on routine cytology and histology and was showing positive CK staining to reach a more confident diagnosis of squamous cell carcinoma by immunocyto-chemical staining.

Thus it's worth noting that from aspirate of different sites we demonstrated the high sensitivity and the broad spectrum association of CK for the diagnosis of malignancy. A prospective study with clinical and histological correlation of these markers would be very helpful to definitely establish the place of these markers in the strategy for the management of aspirate especially in resource poor settings where to apply a large panel of markers for confirmatory diagnosis is a limitation that must be kept in mind when using this reagent. If employing cytokeratin AE1/AE3 as a sole marker of epithelial differentiation as part of a diagnostic panel of immunostains, hepatocellular carcinoma will typically be missed, and a certain percentage of renal, adrenal, and prostate will go unrecognized as epithelial tumors.

Conclusions

1. Cytomorphology remains the most important and reliable diagnostic tool in delineating benign from malignant conditions with particular emphasis on the nuclear morphology of benign and malignant cells.
2. Cytokeratin has 66% sensitivity as a tumor marker in cytologically positive cases of squamous cell carcinoma.

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