Immunohistochemical Expression of Cytokeratin 5/6 in Gynaecological Tumors.

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Citation

Abstract
Gynecological malignancies are very common as they represent second most common cause of fatal cancer in women comprising 42.5% of all malignancies in women. Histopathological examination is helpful to a larger extent in diagnosis of all gynecological malignancies but has limitations in cases of undifferentiated malignancies and tumors showing heterogeneous composition. In such cases immunohistochemistry is strongly recommended. Various tumour markers applied for specific diagnosis are cytokeratin, vimentin, carcinoembryonic antigen (CEA), Epithelial membrane antigen (EMA). Cytokeratin 5/6 being present in cervical epithelium show strong positivity for squamous cell carcinoma and hence preferred. Focal positivity of cytokeratin 5/6 is also seen in ovarian and endometrial adenocarcinoma in areas of squamous differentiation. In the present study expression of cytokeratin 5/6 positivity was evaluated in cervical, ovarian and endometrial carcinoma using primary antibody cytokeratin 5/6; clone D 5/16 B4 (M7237) (DAKO) in formalin fixed and paraffin embedded tissue sections. Results were be be correlated with histological types and different grades.

INTRODUCTION
Neoplasms of the female genitalia represent the second most common source of fatal cancer in women. Gynaecological malignancies comprise 42.5% of all malignancies in women. Carcinoma cervix is the most common malignant tumour found among Indian women constituting 80% of all gynaecological tumours. Ovarian and endometrial tumour constitute 15% and 2% respectively. Generally gynaecological malignancies arises above the age of 35 years with endometrial carcinoma having predilection for elderly women.

Histopathology, although has been helpful to a larger extent in differentiating the type of malignancy and identifying the tissue of origin but it has limited role in undifferentiated malignancies and tumors showing heterogeneous composition. To overcome that immunohistochemistry has a key role in diagnosing malignancies of uncertain origin.

Much divergence is seen in differentiation of epithelium in various organs of female genital tract. Epithelial neoplasm arising from these organs show very heterogeneous and pleomorphic composition. But during malignant transformation of epithelium, its keratin profile remains constant hence keratin expression is widely used in fingerprinting of various carcinomas.

Cytokeratin 5/6 is used as a marker of squamous differentiation and is helpful in assessing poorly differentiated squamous cell carcinoma as well as the squamous differentiation which occur in 20% endometrial adenocarcinoma by its focal positivity.

In addition, Cytokeratin 5/6 has a pivotal role to play in identifying metastatic carcinoma with squamous differentiation such as adenosquamous cell carcinoma of endometrium and ovary.

AIMS AND OBJECTIVES
To study the expression of cytokeratin 5/6 in various histological types and grades of carcinoma cervix, ovarian carcinoma and endometrial carcinoma.

MATERIALS AND METHODS
The study was conducted on 40 cases of gynaecological tumors which included 20 cases of cervical carcinoma, 10 cases of endometrial carcinoma and 10 cases of ovarian carcinoma. Tissues were processed routinely and stained with haematoxylin and eosin (H & E). Cytokeratin 5/6 immunohistochemical staining was done on 4 µm thick paraffin sections using the standard peroxidase-antiperoxidase method. All steps were carried out in a moist and humid chamber, so that the sections remain moist.
throughout the procedure. Positive control was put up with every batch. The primary antibody used was Concentrated mouse monoclonal antihuman cytokeratin 5/6, clone D 5/16 B4 (M7237) (DAKO).

Cytokeratin 5/6 scoring was done according to the criteria laid down by Smedts et al (1992) and Ordonez (1998) in their respective studies on gynaecological malignancies. The scoring was based upon the percentage of CK 5/6 positive cells as score 1 is given when 1-25% of the tissue shows positivity, score 2 when 26-50% was positive, score 3 when 51-75% was positive and score 4 when 76-100% cells show positivity.

RESULTS
Of the forty cases of gynaecological malignancies, 20 were cervical carcinomas which were further differentiated into their histological types and the comparative CK 5/6 expression was studied. Grading was done based upon the criteria laid down by Kristensen B et al on the basis of degree of keratinization, nuclear pleomorphism, pattern of invasion and host response into grade I to grade IV.

Table I shows cervical carcinomas statistics according to histological typing. It was observed that maximum no. of cases were histologically squamous cell carcinoma (large cell non-keratinizing type) and of grade III. Table II shows compiled view of all the cases and IHC scoring for all grades and types.

Table I: Different histological types of cervical carcinomas.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma (Large cell non-keratinizing type)</td>
<td>11</td>
</tr>
<tr>
<td>Squamous cell carcinoma (Large cell keratinizing type)</td>
<td>6</td>
</tr>
<tr>
<td>Squamous cell carcinoma (Small cell type)</td>
<td>2</td>
</tr>
<tr>
<td>Adeno-squamous carcinoma cervix</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
</tr>
</tbody>
</table>

Figure 2
Table II: Correlation of CK 5/6 score with different histological types and grades of cervical carcinomas.

Figure 3
Fig. 1- Strong ck5/6 immunostaining in SCC (large cell keratinizing type) grade I (10x)
Immunohistochemical Expression of Cytokeratin 5/6 in Gynaecological Tumors.

Figure 4
Fig.2-Strong focal ck5/6 immunostaining in SCC (keratinizing type)grade I(10x)

Figure 6
Fig.4. Weak ck5/6 immunostaining in squamous cell carcinoma with minimum keratinization grade 111 (10x)

Figure 5
Fig.3-Moderate ck5/6 immunostaining in squamous cell carcinoma with minimum keratinization grade iii (10x)

CK 5/6 IN ENDOMETRIAL CARCINOMA

Out of the 10 cases of endometrial carcinomas, 6 cases (60%) were positive for CK 5/6. Here also histological typing and grading was done and CK 5/6 expression in all cases was done and results are depicted in table III and IV.

Figure 7
Table 111: Correlation of CK 5/6 score with different histological types of endometrial carcinoma.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>0% stained cells</th>
<th>Score 1 (0-25%)</th>
<th>Score 2 (26-50%)</th>
<th>Score 3 (51-75%)</th>
<th>Score 4 (76-100%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial adenocarcinoma</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Endometrial adenoacanthoma with squamous metaplasia</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Papillary adenocarcinoma endometrium</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Total</td>
<td>4 (40%)</td>
<td>3 (30%)</td>
<td>3 (30%)</td>
<td>-</td>
<td>-</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

Figure 8
Table IV: Correlation of CK5/6 score with histological grades of endometrial carcinoma.

<table>
<thead>
<tr>
<th>Histological grades</th>
<th>Score 1 (1-25%)</th>
<th>Score 2 (26-50%)</th>
<th>Score 3 (51-75%)</th>
<th>Score 4 (76-100%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Well differentiated)</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>II (Moderately differentiated)</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>III (Poorly differentiated)</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td>3 (30%)</td>
<td>3 (30%)</td>
<td>-</td>
<td>-</td>
<td>6 (60%)</td>
</tr>
</tbody>
</table>
**Figure 9**
Fig.5-Weak ck 5/6 immunostaining in endometrial adenocarcinoma (40x)

**Figure 10**
Fig.6-Moderate ck 5/6 immunostaining in poorly differentiated endometrial adenocarcinoma grade 111 (10x)

**Figure 11**
Fig.7-Strong focal ck5/6 immunostaining in areas of squamous and columnar differentiation in endometrial adenocarcinoma with squamous metaplasia (10x)

**Figure 12**
Table V: Correlation of CK5/6 score with histological types of ovarian carcinomas.

<table>
<thead>
<tr>
<th>Histological Type</th>
<th>CK 5/6</th>
<th>Scoring of CK 5/6 positive cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
<td>Score 1 (1-25%)</td>
<td></td>
</tr>
<tr>
<td>Papillary serous cystadenocarcinoma</td>
<td>4</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Mucinous cystadenocarcinoma</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

**Figure 13**
Fig.8- Moderate ck5/6 immunostaining in Papillary serous cyst adenocarcinoma (10x)

**Figure 14**
Table VI: Correlation of CK5/6 score with histological grades of ovarian carcinoma.

<table>
<thead>
<tr>
<th>Histological grades</th>
<th>Scoring of CK 5/6 positive cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Score 1 (1-25%)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Score 2 (26-50%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Score 3 (51-75%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Score 4 (76-100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2 (20%)</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**
The heterogeneous and pleomorphic neoplasms of the female genital tract arising from the epithelia of multipotent coelomic origin are to be diagnosed with the help of immunomarkers like cytokeratin 5/6. CK 5/6 is a stratification marker and is normally present in stratified...
epithelium, transitional epithelium and mesothelial cells and also in malignant transformation of these epithelia.

All of the 20 cervical carcinomas were positive for CK 5/6 in which 19 were squamous cell carcinomas and only 1 was adenosquamous cell carcinoma. This was in congruence with the studies conducted by Moll et al (1983) and Smedts et al (1992) who observed CK 5/6 positivity in all cases of squamous cell carcinoma. Thus, IHC scoring for CK 5/6 was very helpful in analysing large cell keratinizing type squamous cell carcinomas that scored the highest (5 out of 6 cases scored 4) as compared to large non keratinizing type (6 out of 11 cases scored 4) and adenosquamous carcinomas which were weakly positive. This weak expression of CK 5/6 by the glandular architecture in adenosquamous carcinoma cervix favours the fact that adenocarcinoma as well as squamous cell carcinoma of cervix are the part and parcel of the same spectrum but on the opposite ends arising from the common progenitor cells i.e. reserve cells of the transitional zone.

Among the different grades, grade I showed high percentage of CK 5/6 cells with strong intensity as compared to grade IV. It can be assumed that undifferentiated cells possibly have lost its keratin and became larger cells and showed only focal positivity in areas of squamous differentiation.

Out of the 10 cases of endometrial carcinomas, 6 were positive for CK 5/6. Four of these cases pure endometrial adenocarcinoma showed weak positivity for CK 5/6. But comparatively the poorly differentiated endometrial carcinomas showed increased CK 5/6 positivity. Chu et al (2002) and Moll et al (1991) demonstrated that CK 5/6 expression in endometrial adenocarcinoma is focal, weak or not at all, which correlates completely with observation in the present study. Two cases out of six positive cases were having squamous metaplasia which showed greater CK 5/6 expression. Similarly Moll et al (1991) and Hans et al (2003) illustrated CK 5/6 expression in cases of endometrial adenocarcinoma with squamous metaplasia. All the cases were not only positive but also scored high.

Taking into account, 4 positive cases of 10 ovarian carcinomas, Papillary serous cystadenocarcinomas presented with enhanced CK 5/6 expression in comparison to mucinous cystadenocarcinomas irrespective of their grades. This observation correlated completely with study done by Moll et al (1991) on 6 cases of serous cystadenocarcinoma and 2 cases of mucinous cystadenocarcinoma ovary. Both the cases of mucinous cystadenocarcinoma ovary were considered negative for CK5/6. But 5 out of 6 cases of papillary serous cystadenocarcinoma showed focal and irregular immunoreactivity for CK5/6 and scored 1.

Ordonez (1998) studied CK5/6 immunoreactivity in 30 cases of ovarian carcinoma, out of which 10 were positive for CK 5/6. Eight out of these ten positive cases were serous cystadenocarcinoma ovary, among which 7 cases showed low percentage of CK5/6 positive tumour cells and hence scored 1. Only one case showed 30% of tumour cells positive for CK5/6 thus scored 2.

**CONCLUSION**

It is therefore concluded that Cytokeratin 5/6 is the diagnostic marker of squamous differentiation where histologically poorly differentiated or undifferentiated squamous cell carcinomas are concerned.

The focal and weak positivity of CK 5/6 in carcinomas of female genital tract as compared to other adenocarcinoma like that of stomach, kidney, colon which are negative for CK5/6 could be a valuable aid in diagnostic dilemmas of metastasis.

**References**

11. Ordonez NG. Value of cytokeratin 5/6 immunostaining in distinguishing epithelial mesothelioma of the pleural from
Immunohistochemical Expression of Cytokeratin 5/6 in Gynaecological Tumors.

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