Immunohistochemistry in Ovarian Malignancies: A Comparative Study in Two Different Time Eras

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Background: To assess role of immunohistochemistry in identifying primary and secondary ovarian malignancies.

Methods: In this descriptive cross sectional study 65 cases of ovarian malignancies diagnosed during the year 2001, with a limited immunohistochemistry support were included. An equal number of cases (65 cases) diagnosed during the year 2012, with an extended immunohistochemistry support, were used for the comparison. Immunostains used in these cases were CK, CK7, CK20, Cdx2, PLAP, WT1, inhibin, Calretenin, Melan A, CD99, GCDFP15, Mammoglobin LCA, CEA, AFP, CD30 and CD10.

Results: The frequency of metastatic carcinoma has changed tremendously being 3% in 2001 and 35.4% in 2012. The frequencies of other malignancies in 2001 in descending order of frequency were surface epithelial tumour (74.2%), germ cell tumours (16.2%), sex cord stromal tumours (1.5%) and others (3%). In 2012, apart from metastatic carcinoma, the frequency in descending order of frequency was surface epithelial tumours (35.4%), germ cell tumours (18.5%), sex cord stromal tumours (16.2%), and others (4.6%). There was not much difference in median ages (42.74 ±15.4 and 39.54 ±15.8).

Conclusion: IHC has helped in identifying primary and secondary ovarian malignancies.

Key Words: Ovarian malignancy, immunohistochemistry

Introduction

Ovarian cancer is one of the most lethal and common malignancies of females. About 21,880 women were diagnosed and 13,850 women died of ovarian cancer in 2010. 1 A total of 45,000 women were diagnosed with ovarian cancer in 2008 in Europe. 2 Although there are no known definite causes of ovarian malignancy, there are some risk factors. 3 Nulliparity, obesity, women taking hormone replacement therapy for more than five years increase the risk of ovarian cancers. 4 Moreover women harbouring BRCA1 and BRCA2 mutations are at increased risk. 5 Pathogenesis of ovarian carcinoma has been subject of interest in the recent years. Different pathogenesis of ovarian malignancies has important implications regarding the prognosis. 6 It is very important to accurately classify and diagnose ovarian tumours due to different treatment options. Usually tumours with typical morphological features do not pose any diagnostic difficulty, but not uncommonly they share similar patterns. Pattern recognition is very important. At times, morphology alone is not sufficient and one has to rely on ancillary studies. Immunohistochemistry (IHC) over the years has developed as a very helpful diagnostic adjunct. It helps to classify tumours into surface epithelial, germ cell, sex cord stromal tumours and metastatic tumours. It has played a very important role in differentiating primary ovarian malignancies from tumours metastatic to ovary. The role of IHC has very important treatment and prognostic implications.7 No single immunostain is diagnostic. Usually a panel of immunostains has to be applied to reach a final diagnosis. IHC has played a vital role in differentiating between primary ovarian carcinomas from metastasis from colon, breast, pancreas, appendix etc. 6, 7 The rationale of this study was to highlight the value of IHC in identifying primary and secondary ovarian malignancies.

Patients and Methods

This was a descriptive cross sectional study carried out in department of Pathology, Shaukat Khanum Memorial Cancer Hospital and Research centre. The department receives specimens from all over the country and shares a major burden of cancer diagnosis. A total of 65 cases of ovarian malignancies diagnosed during the year 2001 were randomly selected. This was the time when immunohistochemistry used to be applied on a very limited scale. In 2001 immunostains which used to be applied were Cytokeratin (CK), Leucocyte common antigen (LCA), Inhibin, CK7 and Placental Alkaline Phosphatase (PLAP). Mean, median and mode were calculated for quantitative variable like patient’s age and frequencies and percentages were calculated for tumour type. An equal number of cases (65 cases)
diagnosed during the year 2012 were used for the comparison and same variables were calculated. Now we use extended panel of immunohistochemistry for cancer diagnosis. Immunostains used in these cases were CK, CK7, CK20, Wilms tumour 1 (WT1), Caudal type homeo box transcription factor 2 (Cdx2), PLAP, inhibin, Calreteinin, MelanA, Cluster of differentiation 99 (CD99), Gross cystic disease fluid protein 15 (GCDFP15), LCA, Carcinoma embryonic antigen (CEA), Alpha fetoprotein (AFP), and Cluster of differentiation 10 (CD10) and Cluster of differentiation 30 (CD 30). Results obtained for the year 2001 and 2012 were compared to see if immunohistochemistry has played any role in identifying primary and secondary malignancies of ovary.

**Results**

Median ages of patients, diagnosed with ovarian malignancies, during the years 2001 and 2012, were in approximation, i.e., 42.74 ±15.4 during 2001 and 39.54 ±15.8 during 2012. In 2001, out of 49 cases of surface epithelial tumours, 81.6% cases presented in 4th to 6th decades. A total of 10 out of 12 cases (83.3%) of germ cell tumours presented in 2nd decade. One case of metastatic carcinoma presented in 4th and one in 5th decade. In 2012, 17 out of 23 (74%) surface epithelial tumours presented in 2nd to 3rd decade, 9 out of 12 cases (75%) of germ cell tumours in 2nd to 3rd decade and 9 out of 23 cases of metastatic carcinoma (39%) in 4th decade and five cases (21%) each in 5th and 6th decade. In 2001 metastatic tumours were 3%, but in 2012 frequency of metastatic tumours escalated to 35.4%. Surface epithelial tumours constituted 74.2% in 2001, but in 2012 their frequency was noted 35.4%(Table 1). Among surface epithelial tumours, serous papillary carcinoma remained the commonest in 2001 and 2012(Table 2). Among germ cell tumours, dysgerminoma comprised 61.5% of the cases, followed by yolk sac tumours and mixed germ cell tumours (15.4% each). In 2012 most common germ cell tumour was dysgerminoma (58.4%), followed by immature teratoma (16.7%), mixed germ cell tumours (16.6%) and yolk sac tumor (8.3%). There was only one (1.5%) adult granulosa cell tumour diagnosed in sex cord stromal tumour category in 2001 and four adult granulosa cell tumours (6.2%) in sex cord stromal category in 2012.

In 2001, there were only two cases (3%) of metastatic carcinoma, one metastasis from gastrointestinal tract and other from breast. In 2012, there were 23 cases (35.4%) of metastatic carcinoma. Among the latter, 2 cases had metastasis from uterus, 12 from GIT (fig 3, 4, 5), 2 from breast (fig 1and 2) and 1 from kidney. Six cases had ovarian metastasis of unknown origin. Origin could not be determined even after application of an extensive panel of antibodies. Immunopanel used in 2001 was fairly smaller than what we use in 2012 (Table 3).

### Table I: ovarian tumours in 2001 and 2012

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>2001</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface epithelial tumours</td>
<td>48 (74.2%)</td>
<td>23 (35.4%)</td>
</tr>
<tr>
<td>Germ cell tumours</td>
<td>12 (18.3%)</td>
<td>12 (18.4%)</td>
</tr>
<tr>
<td>Sex cord stromal tumours</td>
<td>2 (3%)</td>
<td>23 (35.4%)</td>
</tr>
<tr>
<td>Metastatic (Total)</td>
<td>1 (1.5%)</td>
<td>4 (6.2%)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>2 (3%)</td>
<td>3 (4.6%)</td>
</tr>
</tbody>
</table>

### Table 2: Histological types of surface epithelial tumours in 2001 and 2012

<table>
<thead>
<tr>
<th>Surface Epithelial Tumors</th>
<th>2001</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous carcinomas</td>
<td>33(61.1%)</td>
<td>42 (65.3%)</td>
</tr>
<tr>
<td>Mucinous carcinomas</td>
<td>12 (18.4%)</td>
<td>3 (4.3%)</td>
</tr>
<tr>
<td>Endometrioid carcinomas</td>
<td>11 (16.3%)</td>
<td>14 (21.7%)</td>
</tr>
<tr>
<td>Clear cell carcinomas</td>
<td>9 (14.2%)</td>
<td>6 (8.7%)</td>
</tr>
</tbody>
</table>

### Table 3: Immunopanel used for diagnosis of primary and secondary ovarian malignancies in 2001 and 2012

<table>
<thead>
<tr>
<th>Immunopanel available in 2001</th>
<th>Surface epithelial tumours</th>
<th>Germ cell tumours</th>
<th>Sex cord stromal tumours</th>
<th>Metastatic tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK, CK7, LCA</td>
<td>CK, CK7</td>
<td>PLAP</td>
<td>Inhibin</td>
<td>CK, CK7, LCA</td>
</tr>
</tbody>
</table>

**Discussion**

Ovarian malignancies rank among the ten most common malignancies in Pakistani women. We have compared frequencies of ovarian malignancies diagnosed during the year 2001 when a very limited panel of immunostains was available and 2012 when extensive panel of immunostains was available. Special emphasis was given in to the role of immunohistochemistry in identifying primary and secondary malignancies. Presumably, the frequency of metastatic carcinoma in ovary has changed after the use of IHC. In 2001 the most common tumours were surface epithelial tumours (74.2%). Results were similar to some Pakistani studies. In all the later Pakistani studies, IHC was not used. The percentage of surface epithelial malignancies dropped from 74.2%
Fig 1: Metastatic carcinoma of breast origin; Fig 2: Metastatic carcinoma of breast origin showing GCDFP-15 expression (200x)

Fig 3: Metastatic colorectal adenocarcinoma (400x); Fig 4: Metastatic colorectal adenocarcinoma showing cdx2 expression (100x)

Fig 5: Metastatic colorectal adenocarcinoma showing CK20 expression (100x)

to 35.4% in 2012. This change has resulted from the increase in percentage of metastatic carcinomas after the use of IHC technique. In present study commonest epithelial malignancy was serous carcinoma in both years (2001 and 2012). In 2001, serous carcinoma was followed by mucinous, endometrioid and clear cell carcinomas in decreasing order of frequency, whereas in 2012 serous carcinoma was followed by endometrioid adenocarcinomas, clear cell carcinomas and mucinous carcinomas. The results of 2001 were comparable with the studies by Ahmed et al, Tanwani et al, showing serous carcinomas commonest, followed by mucinous and endometrioid carcinomas. On the other hand in studies by Jamal et al and Khan et al serous carcinomas were most common followed by endometrioid and mucinous carcinomas. In studies by Muzaffar et al and Iltaf et al mucinous carcinomas were more common than serous carcinomas. On the whole the important point was that, mucinous carcinoma occupied the second place in 2001 in our study and second or first place in local studies which we compared. In 2012, mucinous tumours dropped to fourth place. It can be ascribed to an increase in detection rates of metastatic adenocarcinomas after using extensive panel of IHC. The role of IHC in differentiating primary mucinous carcinoma and mucinous carcinomas from gastrointestinal tract is limited; nevertheless the combination of light microscopic findings, IHC and radiological and clinical parameters help in differentiating between the two in most of the cases. Germ cell tumours were the second most common group in our study in 2001. Results were similar to local studies. Germ cell tumour was second most common tumour (15-20%) in few western studies by Greene and Wooster et al. An important finding in our study was that the metastatic carcinomas shared the similar percentage (35.4%) as that by surface epithelial tumours in 2012. Metastatic group constituted only a small percentage in other studies like Khan et al (1%) and Iltaf et al (0.7%). In various local studies done on frequencies of ovarian malignancies like studies by Saeed et al, Muzaffar et al, Jamal et al and Ahmed et al, metastatic carcinoma groups constituted a very small percentage. International studies, conducted by Yada-Hashimoto, Kir and Khunamornpong et al, using IHC panels, revealed the frequency of metastatic tumours from 17.4% to 30%. These higher percentages are similar to present study. All these studies had gastrointestinal tumours as the most common source of ovarian metastasis, substantiating the present study. In another study by de Waal et al, metastatic ovarian carcinomas constituted 15% of ovarian malignancies. This number was lower as compared to that of our study. In another study by Moore RG et al colon cancer was the most common source of ovarian metastasis (32.2%), followed by appendix (20.3%), breast (8.4%) and others. Most primary source of ovarian metastasis in our study was also GIT. Results were different in study by Skírnisdóttir I et al in which the most common source of ovarian metastasis was breast carcinoma followed by GIT. Our study had opposite results. Main reason for different results may be geographical variations, selection of cases and different expertise of different tertiary care hospitals.

There is a tendency of metastatic carcinomas being misdiagnosed as one of the primary surface epithelial tumor, if IHC techniques are not used or a limited panel is used. The later phenomenon applies mostly to mucinous tumors which turn out to be metastasis from appendix, intestine or pancreas etc. When tumor comprises predominantly of signet ring cells, it is required, by applying IHC, to rule out metastasis from breast and gastrointestinal tract before labeling it as primary ovarian carcinoma. Generally panel used in our setup to differentiate between primary ovarian carcinomas from metastatic carcinoma is: CK7, CK20, WT1, ER, PR, GCDFP-15, Mammoglobin, CEA, Cdx2 and CA 125. CK7 and CA125 are positive in primary
Overlapping IHC results sometimes positive with CK20 and CDX2 and negative with CK7. Parameters play an important role to rule out metastasis from breast. Panel which is used to differentiate primary endometrioid adenocarcinoma from metastatic colonic carcinoma is: CK7, CK20, ER, PR, CA125, and Cdx2. Primary endometrioid ovarian carcinomas are positive for CA125, CK7, ER, PR but usually negative for CK20 and Cdx2. Reverse is true for metastatic colonic adenocarcinomas. A panel of CK7, CA125, CK20, Cdx2, ER and PR is used to differentiate between primary mucinous and metastatic colon carcinoma. Metastatic large intestinal adenocarcinomas are positive for CK20 and cx2 and negative for CK7 and CA125. Primary mucinous carcinomas of ovary are positive for CA125 and more diffusely positive for CK7 than CK20. They can also be positive for CDX2. Metastatic carcinomas from upper GIT and pancreaticobiliary origin are usually positive for CK7 and CDX2 and negative for CK7 and CA125. However, these results are not absolute. There is lot of overlapping. Both primary mucinous carcinomas from ovary and metastatic carcinomas from GIT can be positive with CK20 and CDX2 and negative with CK7 and CA125. Overlapping IHC results some times makes it difficult to differentiate between primary mucinous carcinomas of ovary and metastasis for GIT. Then, the combination of clinical history, light microscopic, immunohistochemical and radiological parameters play an important role.

**Conclusion**

IHC leads towards making an accurate diagnosis, thereby translating into better management options.

**References**