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WT1, Bcl-2, Ki-67 and Her2/Neu as diagnostic and prognostic immuno markers in ovarian serous and endometrioid carcinoma

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Abstract
Background: Ovarian cancer is the fifth common cause of death due to cancer in women. It constitutes 3% of all cancers in females and 15-20% of genital malignancies. Most of the ovarian cancers are serous type followed by endometrioid type. Sometimes glands of these two carcinomas are indistinguishable histologically. It also becomes difficult to differentiate these two types when they are poorly differentiated.
Aims: The aim of this study was to find out differences in immuno-markers expressions between serous and endometrioid carcinomas and association of their staining patterns with other clinico-pathological prognostic factors.
Materials and Methods: Immunohistochemical staining for WT1, Bcl2, Ki67 (MIB1) and Her2/Neu were done in paraffin embedded tissues of histologically diagnosed total 38 cases (21 serous and 17 endometrioid) of ovarian carcinomas and staining patterns were correlated with other clinico-pathological prognostic factors.
Results: Out of these 38 cases, 24 cases were in stage I/II (early stage) and 14 cases stage III/IV (advance stage). On the other hand, 16 cases were low grade, 12 cases intermediate grade and 10 cases high grade. Twenty out of 21 serous carcinomas were positive for WT1, whereas most of the endometrioid carcinomas were negative. Ki67 labeling index and Her2/Neu were higher in both higher grade and stages. On the contrary, intensity of Bcl2 staining was lower in higher grades and stages lesions.
Conclusions: Use of WT1 may be useful in resolving diagnostic dilemma between serous and endometrioid carcinoma, especially in difficult cases. Ki67, Bcl2 and Her2/Neu may be used as prognostic markers.

Keywords: Ovarian carcinoma, serous carcinoma, endometrioid carcinoma, immunohistochemistry

Introduction

Ovarian tumors accounts for 3% of all cancers in females and fifth most common cause of death due to cancers in women [1]. Among the primary ovarian tumors, 80% are epithelial tumors, which mostly consists of serous, mucinous and endometrioid types [1]. Serous carcinoma is the most common malignant ovarian tumor, followed by endometrioid carcinoma [1]. The histological features of poorly differentiated endometrioid carcinoma sometimes merge almost imperceptibly with those of poorly differentiated serous carcinoma, making the histological diagnosis difficult [2].

Ovarian serous carcinomas demonstrate diffuse strong nuclear expression of WT1, a tumor suppressor gene, which is usually negative in endometrioid carcinoma [3]. The nuclear WT1 protein expression can identify misclassified high-grade endometrioid carcinomas [2]. Bcl2, an anti-apoptotic protein, encoded by the BCL2 gene, regulates apoptosis [4]. Study found that Bcl2 expression correlates significantly and decreasing patient survival parallels the decreased expression in ovarian tumor [5]. Mitotic count is a traditional and practical method to determine proliferative activity, but is hampered by several ineluctable factors [6]. Immunohistochemistry for Ki67 is an alternative way and has become a widely accepted method. High expression of Ki67 has been found to indicate a poor prognosis in several cancers, including ovarian cancers [7]. Her2/Neu protein is encoded by ERBB2, seen amplified in different cancers including adenocarcinoma of ovary [1].

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In a study, high survival rate was found in patients without Her2/Neu in endometrioid tumor [8].

Materials & Methods
The study included total 38 histologically diagnosed cases of serous carcinoma (21 cases) and endometrioid carcinoma (17 cases). Institutional ethical clearance was taken. All the relevant history and operative findings were recorded and International Federation of Gynecology and Obstetrics (FIGO) staging was done. Histologically serous carcinoma was divided in three grades as proposed by Shimizu M *et al.* [9] Grading of endometrioid carcinoma was done by FIGO Grading Scheme. Immunostaining was done on the representative paraffin embedded tissue sections by standard procedure.
For WT1 staining, the number of tumor cells with nuclear staining and intensity were recorded and classified as 0 (No staining), 1+ (Faint staining), 2+ (Mild to moderate intensity) and 3+ (Strong intensity) [9]. The Ki67 index was defined as percentage of immunoreactive tumor cells out of the total number of tumor cells in most intense staining areas [10]. Bcl2 staining intensity was graded as 0 (No staining), 1+ (Weak staining) and 2+ (Strong staining) [5]. HER2/Neu staining intensity was classified as 0, 1+, 2+ and 3+ and only 3+ staining intensity was considered as positive or over-expression [11].

Results
Nine out of 21 (42.9%) serous carcinoma were bilateral, in contrast to three out of 17 (17.6%) endometrioid carcinoma. The mean age of serous and endometrioid carcinoma were 47.3 year (SD-9.2) & 53.2 year (SD-9.5) respectively. Of the 21 serous carcinomas, eight cases (38.1%) were grade 1, six cases (28.6%) grade 2 and seven cases were grade 3. Among the 17 endometrioid carcinomas, eight cases (47.1%) were grade 1, six cases (35.3%) grade 2 and three cases (17.6%) were grade 3. Nine out of 21 (42.9%) serous carcinoma and five out of 17 (29.4%) endometrial carcinomas presented as advanced stages (Stage III and IV) disease. No significant correlations were found between the age, stages and bilaterality.
Total 20 out of 21 (95.2%) serous carcinoma, irrespective of their age, grades and stages were positive for WT1 staining. Among them, 15 cases (71.4%) showed 3+ positivity and five cases (23.8%) showed 2+ positivity. In contrast, 15 out of 17 endometrioid carcinomas (88.2%) were negative for WT1 staining. Two cases (11.8%) showed 1+ positivity. The differences in the staining pattern was statistically significant (p-value < 0.001) between serous and endometrioid carcinoma. No significant relationship found between stage and grades of tumors and WT1 staining pattern.

A.   B.
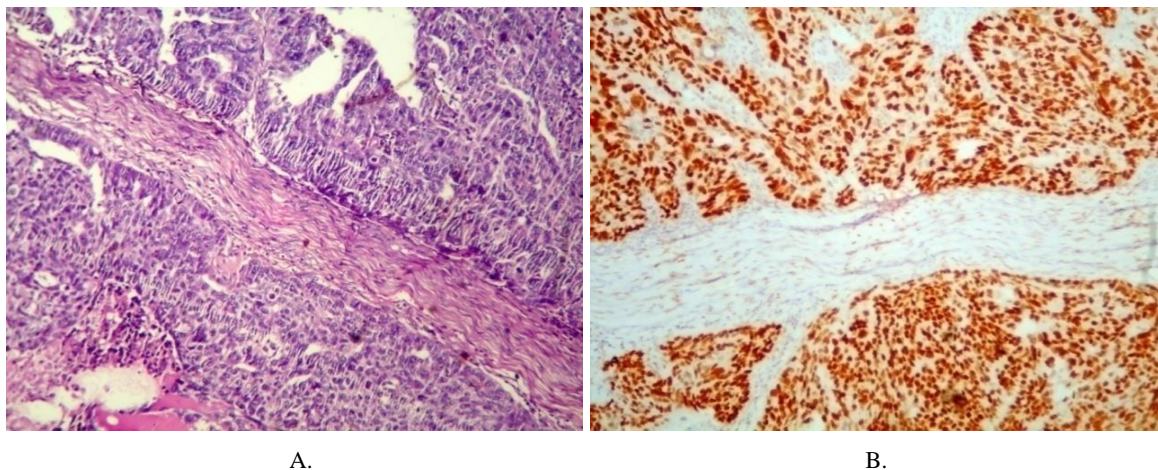
Fig 1: A. High grade serous carcinoma (H&E, 100x) B. WT1 staining, 100x (3+ positivity)

We found 10 out of 16 grade 1 tumors showing 2+ intensity for Bcl2. Five cases showed 1+ intensity and one case was negative. In 12 intermediate grade tumors, five cases showed 2+ intensity, five cases showed 1+ intensity and two cases were negative. In 10 high grade tumors, only one case showed 2+ intensity, three cases showed 1+ intensity and six cases were negative. This pattern of Bcl2 expression is statistically significant between the low grade and high grade lesions (p-value 0.016). In 14 of the 24 early stage carcinoma Bcl2 showed 2+ positivity, nine cases showed 1+ intensity and one case was negative for Bcl2. In contrast, 8 of the 14 advanced stages diseases were negative for Bcl2. Five cases showed 1+ intensity and one case showed 2+ intensity. The intensity of Bcl2 staining was significantly lower in advanced stages lesions than the early stages lesions (p-value < 0.001). However we found no statistically significant differences between the types of the tumors and Bcl2 staining pattern.
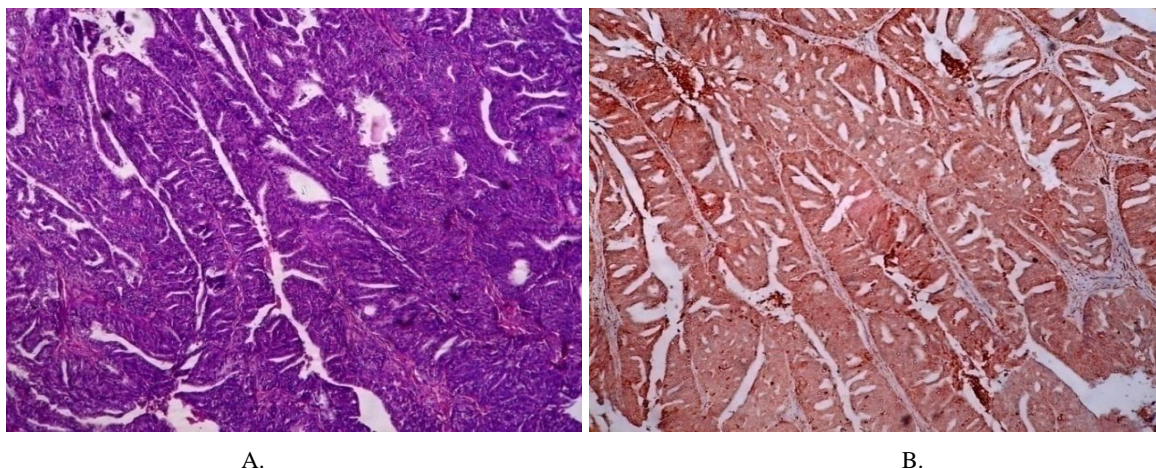
Fig 2: A. Intermedate grade endometrioid carcinoma (H&E, 100x) B. Staining for Bcl2, 100x (2+ positivity)

Mean Ki67 index in low grade lesions was 9.89% (SD-3.50). In intermediate grade and high grades lesions it was 20.39% (SD-8.12) and 41.17 (SD-7.01) respectively. The difference is statistically significant between low grade and high grades lesions (p-value 0.010) and also between low grades and intermediate grades lesions (p value 0.001). However no significant difference of mean Ki67 index was found between intermediate grade and high grades lesions. The mean Ki67 index was 13.78% (SD-8.10) in the early stages and 34.69% (SD-11.52) in advance stages, the difference being statistically insignificant (p-value 0.135).

No significant difference in mean Ki67 index was found between serous and endometrioid carcinomas.

In this study, ten patients (26.32%) showed HER2/Neu over-expression. It was 12.5% (two out of 16) in low grade, 33.33% (four out of 12) in intermediate grade and 40% (four out of 10) in high grade. In early stages, it was 16.67% (four out of 24) and in advanced lesions it was 42.86% (six out of 14). The difference is not significant between grades (p-value 0.241) or stages (p-value 0.070). No significant difference found between HER2/Neu over-expression and types of tumors.
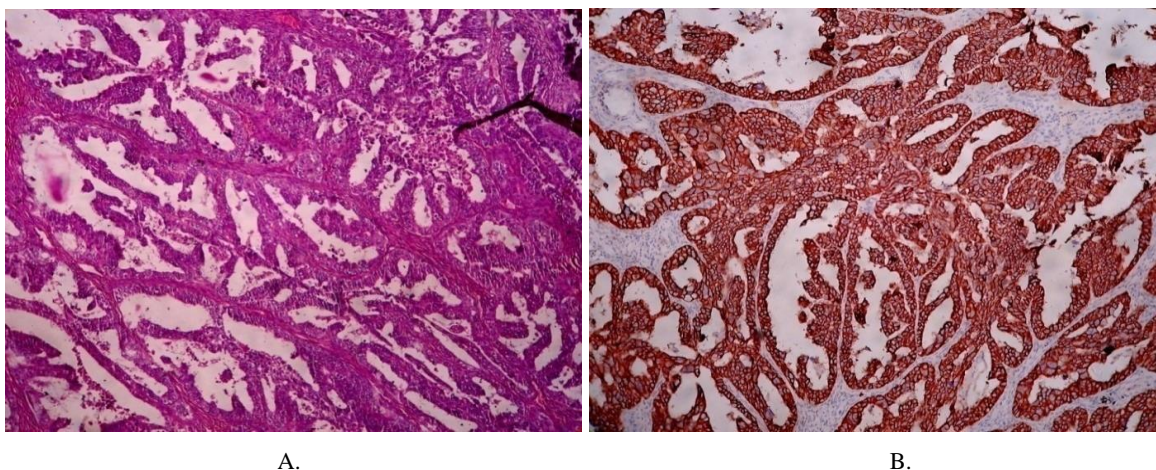
Fig 3: A. High-grade serous carcinoma (H&E, 100x) B. HER2/Neu staining 100x (3+ positivity)

Discussions
The findings of WT1 staining are in concordance with other studies. In their study of 500 ovarian tumors of different types, Kobel M. *et al*. [12] found more than 75% positivity of serous carcinoma for WT1 in contrast to less than 10% endometrioid carcinoma. Hwang H. *et al*. [13] found WT1 positivity in 93% cases of serous carcinoma whereas all the endometrioid carcinomas were negative. We could not found any relation between WT1 expression and stages and grades of the ovarian tumors. In contrast, the study by Netinatsunthorn W. *et al*. [14] suggests that expression of WT1 gene may be indicative of an unfavorable prognosis in advanced serous carcinoma. Another study by Yamamoto S. *et al*. [15] found that a high WT1 immuno-reactivity were associated with higher grade ovarian serous carcinoma and poorer clinical outcome.

Our findings of Bcl2 staining are similar with that of Anderson NS *et al*. [16] Similarly, other studies have reported an inverse relationship between epithelial Bcl 2 expression and tumor grade [17, 18] In contrast, O'Neill CJ *et al*. [19] showed statistically significant higher Bcl2 expression in higher grades lesions. None of the above mentioned studies found any significant correlation between Bcl2 expression and types of the tumors.

In a study of 68 ovarian carcinomas, Aune G *et al*. [10] found statistically higher Ki67 index in advanced stage and higher grades lesion in compared to early stage and low grades lesions. They found no significant differences between Ki67 index with that of serous and non-serous carcinoma. These findings are in concordance with other study [20]. In their study of 50 ovarian tumors, Choudhury M. *et al*. [21] found significantly higher Ki67 index in advance stages lesions.

However there was no relation between mean Ki67 index and grades of tumors. We found that the proliferation markers Ki67 was positively correlated to the tumor grades in the group of carcinomas. There was, however, a considerable overlap of indices between the different malignancy groups, which represent a serious drawback of Ki67 immunostaining. Thus, in an individual case, a low index does not necessarily imply a biologically benign tumor, and vice versa.

The percentage of patients with ovarian cancer having HER2/Neu over-expression ranged between 5.9% to 40% [22, 23]. This wide range of prevalence may be explained by variation in the assessment of HER2/Neu activation by immunohistochemical or other methods. Although study reported impaired clinical outcomes in patients with HER2/Neu over-expression, [24] others failed to demonstrate the relation [25]. Berchuck et al. [24] found that the patients who had over-expression of this protein also had more chance to have persistent disease at second-look laparotomy and had drastically reduced median survival, in comparison to those patients who had normal tumor HER2/Neu expression. Tuefferd M et al. [11] found over-expression of HER2/Neu in 6.6% of 320 ovarian tumors and no prognostic value of HER2/Neu was found.

Conclusions
In this study, most of the serous tumors of ovary were strongly positive for WT1, whereas most of the endometrioid tumors of ovary were negative. So in our opinion, use of WT1 immuno-marker may be considered when there is a diagnostic dilemma between serous and endometrioid carcinomas, especially in difficult cases. Bcl2 staining intensity was significantly lower in high grade and advanced stage lesions. Ki67 index was significantly higher in high grade lesions in contrast to the low grade lesions. Lower Bcl2 and higher Ki67 expression point toward aggressive behavior of the ovarian serous and endometrioid tumors. HER2/Neu over-expression was found in 26.32% cases. No statistically significant correlation was found between HER2/Neu over-expression and grades and stages of the serous and endometrioid tumors of ovaries.

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