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Original Research Article

Expression of Wilms tumor gene protein (WT1) in different histologic subtypes of ovarian carcinoma

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ABSTRACT

Background: Ovarian carcinoma is the seventh most common cancer among women worldwide and the eighth leading cause of death. Ovarian cancer develops in the ovary's tissues.
Methods: This cross-sectional descriptive study was carried out at the Department of Pathology, RMC and Department of Pathology, BSMMU, Dhaka, Bangladesh from July 2019 to June 2021 carried out with 31 histopathologically confirmed ovarian carcinomas. All cases were subjected to WT1 immunostaining followed by histopathological examination. The collected data were analyzed using Statistical Package for Social Sciences (SPSS) software, version-23.0. The ethical clearance of this study was obtained from the Institutional Review Board (IRB) of Rajshahi, Medical College, Rajshahi, Bangladesh.
Results: A total of 31 histopathologically confirmed ovarian carcinoma patients were included in this cross-sectional descriptive study. All cases were subjected to WT1 immunostaining followed by histopathological examination. The age of the study sample ranged from 20 to 75 years. The mean age of the respondent was 45.10±13.78 years. According to the histologic subtypes, WT1 was mainly expressed in serous carcinoma. All the serous carcinoma (16/16) showed a positive reaction for WT1. Among them, 14 cases (87.5%) showed >50% (3+) WT1 score and two (2) cases (12.5%) showed 11% to 50 % (2+) scores. Regarding endometrioid carcinoma four (4) (66.6%) of six (6) were negative whereas one (1) (16.6%) showed 1%-10% (1+) and one (1) (16.6%) showed 11%-50% (2+) WT1 scores. All the mucinous (4/4) and all the clear cell carcinoma (3/3) were negative with less than 1 % of the tumor cells stained two (2) cases of undifferentiated carcinoma were also negative. There was a significant relation between WT1 expression and different histologic subtypes of epithelial ovarian carcinoma with a p value of 0.001 ($p<0.05$).
Conclusions: WT1 can be applied by both the histopathologist and clinician as an effective marker regarding diagnosis and patient management.

Key Words: Ovarian, Carcinoma, Histologic, Gene protein

INTRODUCTION

Worldwide Ovarian cancer is the seventh most frequent cancer in women, with malignant surface epithelial-stromal ovarian neoplasms accounting for over 90% of initial ovarian malignancies.[1] WT1 is not expressed in the healthy fallopian tube epithelium, which is assumed to be the genesis of the majority of high-grade serous carcinomas. WT1 immunohistochemistry is commonly utilized for diagnostic purposes in gynecological

pathology. It distinguishes between distinct histological forms of ovarian cancer.[2] Most ovarian malignancies appear to be caused by the loss or modification of tumor suppressor gene activity and expression. The most common events described are changes in the p53 gene.[3] Wilms tumor gene (WT1) was discovered to be a tumor suppressor gene on chromosome 11p13.[4] Unlike most other tumor suppressor genes, WT1 expression in normal human tissue appears to be restricted to the urogenital system and mesoderm-derived tissues.[5] Ovarian carcinoma has the highest aggressively and mortality rate among gynecological cancers when compared to its incidence rate.[6] Ovarian cancer is typically diagnosed in advanced stages (II-IV), which are distinguished by carcinomatous invasion beyond the ovarian surface and pelvis, as well as peritoneal cavity dissemination. Because of advancements in surgery and chemotherapy using empirically adjusted combinations of conventional medicines, the ovarian cancer survival rate remains about 30%.[7] Epithelial ovarian carcinoma is the most frequent form, accounting for 60%-70% of all ovarian carcinomas. Ovarian carcinogenesis has demonstrated that all cancers are derived from the ovarian surface epithelium, with subsequent metaplastic alterations leading to the creation of distinct cell types.[8] Several studies have shown that ovarian carcinoma may start in other pelvic organs, the majority of ovarian malignancies (>85%) arise from the ovary's surface epithelium. Serous (the most frequent histologic subtype), clear cells, endometrioid, mucinous, transitional, and undifferentiated carcinomas are the most prevalent tumor forms. Ovarian cancer frequently spreads to serous cavities, causing malignant peritoneal and pleural effusion.[9] Ovarian Carcinoma metastasis is closely linked to epithelial-mesenchymal transition (EMT), a biological process in which epithelial cells acquire new mesenchymal characteristics, which plays an important role in upregulating the expression of cell motility-related proteins, enhancing cell migration and invasion, and promoting cancer cell metastasis.[10] WT1 expression is indicative of the serous phenotype in ovarian neoplasm, with substantially lower degrees of staining in other morphological subtypes of ovarian cancer. Some researchers have investigated the use of WT1 in histological and cytological materials as a marker of ovarian carcinoma to differentiate it from adenocarcinoma developing elsewhere. Both epithelial ovarian cancer instances are sporadic, with no family history of the disease.[11] The aim of this study was to evaluate the expression of Wilms tumor gene protein (WT1) in different histologic subtypes of ovarian carcinoma.

METHODS

This cross-sectional descriptive study occurred at two notable medical institutions in Bangladesh: Rajshahi Medical College (RMC) in Rajshahi and Bangabandhu Sheikh Mujib Medical University (BSMMU) in Dhaka. The study was conducted from July 2019 to June 2021. The study was carried out with 31 histopathologically confirmed ovarian carcinoma of different subtypes in the Department of Pathology, Rajshahi Medical College. All cases were subjected to WT1 immunostaining followed by histopathological examination. The collected data were analyzed using Statistical Package for Social Sciences (SPSS) software, version-23.0. Descriptive inferential statistical analysis was performed and the results were presented as frequency and percentage in tables and charts. Chi-square tests were performed to compare WT1 expression in different histologic subtypes of ovarian carcinoma where $p<0.05$ considered as the level of significance. The ethical clearance of this study was obtained from the Institutional Review Board (IRB) of Rajshahi, Medical College, Rajshahi, Bangladesh.

Inclusion and exclusion criteria

Histopathologically confirmed ovarian carcinoma patients were included. Clinically suspected patients but not histopathologically confirmed as ovarian carcinoma were excluded.

RESULTS

The (Table 1) showed the age distribution of the patients. The age of the study sample ranged from 20 to 75 years. The cases were grouped based on decades. It was observed that the majority (29.0%) of patients belonged to the age group 51 to 60 years. The next frequent age group was 31-40 years (25.8%). The median age was 47.0 years and the mean age±SD of the respondent was 45.10±13.78 years.

Table 1: Distribution of the subjects according to age (n=31).

Age groups (yrs)	N	%
20 to 30	6	19.4
31 to 40	8	25.8
41 to 50	5	16.1
51 to 60	9	29
61 to Highest	3	9.7

Range: Min=20 yrs, Max=75 yrs; X±SD=45.10±13.78 yrs.

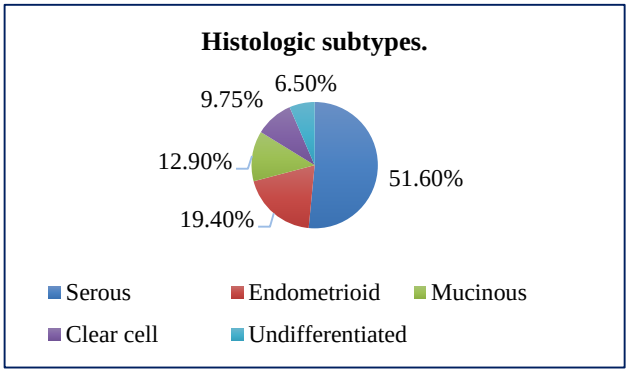

Figure 1: The distribution of histologic subtypes.

The (Figure 1) showed the distribution of histologic subtypes. Among 31 cases serous subtypes were more frequent, a total of 16 cases (51.6 %). The next frequent

subtype was endometrioid, with a total of six cases 6 (19.4%).

Table 2: Distribution of WT1 scores (n=31).

WT1 Scores	N	%
<1% (0 or Negative)	13	41.9
1%-10% (1+)	1	3.2
11%-50% (2+)	3	9.7
>50% (3+)	14	45.2

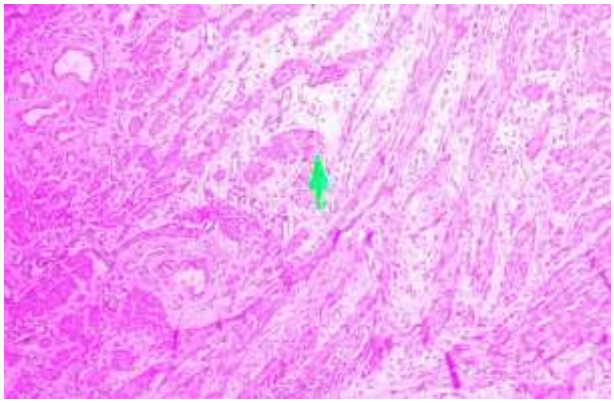

Figure 2: Ovarian serous carcinoma (case no. 06, H & E stain, ×100).

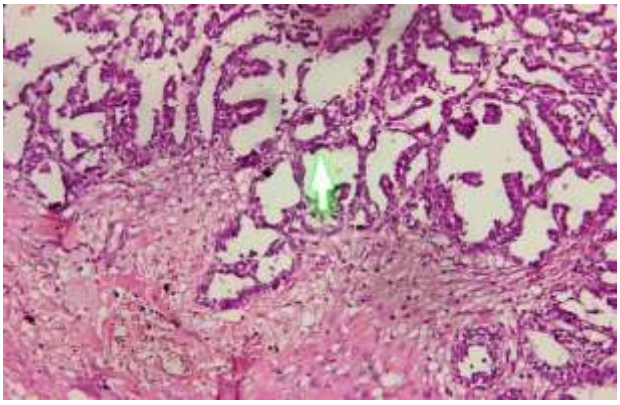

Figure 3: Ovarian serous carcinoma (case no. 06, H & E stain, ×400).

There were a total of four cases 4 (12.9%) of mucinous subtypes. On the other hand, clear cell subtypes, a total of three cases 3(9.7%), and undifferentiated, total of two cases 2(6.5%) were less frequent. The (Table 2) showed the distribution of WT1 scores among all 31 cases. Here most cases, total of 14 cases (45.2%) showed >50% (3+) score and 13 cases (41.9%) showed <1% (0 or negative scores). Only one case 1(3.2%) showed 1% to 10% (1+) scores and three cases 3(9.7%) showed 11 to 50% (2+) scores. The (Table 3) showed the Distribution of WT1 scores in different histologic subtypes of epithelial ovarian carcinoma. A tumor was considered negative if less than 1% of the tumor cells were stained. Positive tumors were graded as follows: 1+, 1% to 10 %; 2+, 11% to 50%; and 3+, >50% positive cells.

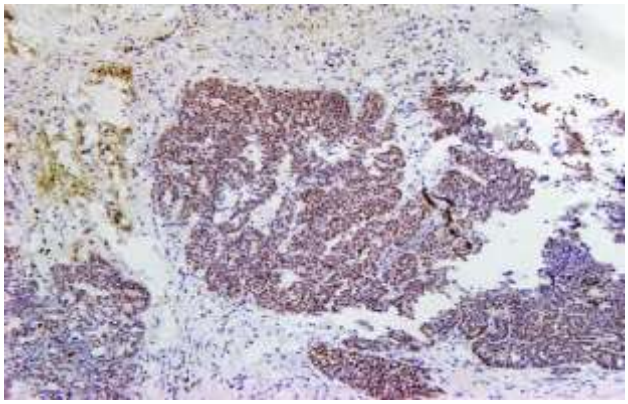

Figure 4: Ovarian serous carcinoma (case no. 10, WT1 stain, ×100).

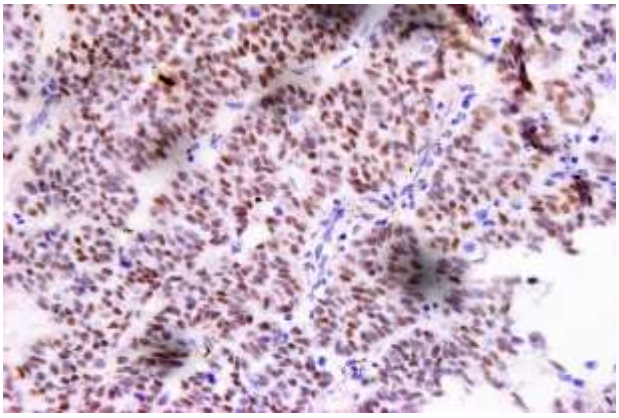

Figure 5: Ovarian serous carcinoma (case no. 10, WT1 stain, ×400).

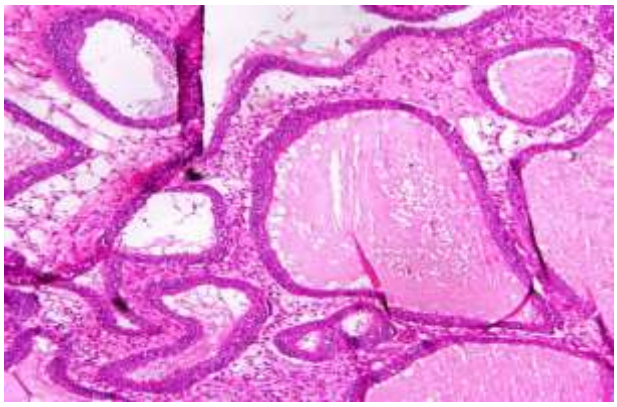

Figure 6: Ovarian mucinous carcinoma (case no-25, H & E stain ×400).

The expression of WT1 was positive in 18 (58.1%) cases, with the remaining 13 (41.9%) cases being negative. There was 16 (51.6%) serous carcinomas out of total of 31 cases and all were positive. All the mucinous four (4) (12.9%) of 31, clear cell three (3) (9.7%) of 31, and undifferentiated two (2) (6.5%) of 31 cases were negative. Among six 6 (19.4%) endometrioid carcinoma two (2) (6.4%) showed positive and four 4(12.9%) showed negative reactions.

Table 3: Distribution of WT1 scores in different histologic subtypes (n-31)

Histologic Subtypes N (%)						
WT1 Scores	**Serous**	**Endometrioid**	**Mucinous**	**Clear Cell**	**Undifferentiated**	**Total**
(0 or negative)	0 (0)	4 (12.9)	4 (12.9)	3 (9.7)	2 (6.5)	13 (41.9)
(1+)	0 (0)	1 (3.2)	0 (0)	0 (0)	0 (0)	1 (3.2)
(2+)	2(6.5)	1 (3.2)	0 (0)	0 (0)	0 (0)	3 (9.7)
(3+)	14(45.2)	0 (0)	0 (0)	0 (0)	0 (0)	14 (45.2)
Total	16 (51.6)	6 (19.4)	4 (12.9)	3 (9.7)	2 (6.5)	31 (100)

x^2=33.41, df=12, p<0.05.

Table 4: WT1 Expression in different histologic subtypes of ovarian carcinoma (n=31).

WT1 Expression					
Positive cases N (%)					**Negative cases N (%)**
Histologic subtype	**No of cases**	**1%-10%**	**11%-50%**	**>50%**	**<1%**
Serous	16	0 (0)	2 (12.5)	14 (87.5)	0 (0)
Mucinous	4	0 (0)	0 (0)	0 (0)	4 (100)
Endometrioid	6	1 (16.6)	1 (16.6)	0 (0)	4 (66.6)
Clear cell	3	0 (0)	0 (0)	0 (0)	3 (100)
Undifferentiated	2	0 (0)	0 (0)	0 (0)	2 (100)

X^2=33.41, df=12, p<0.05.

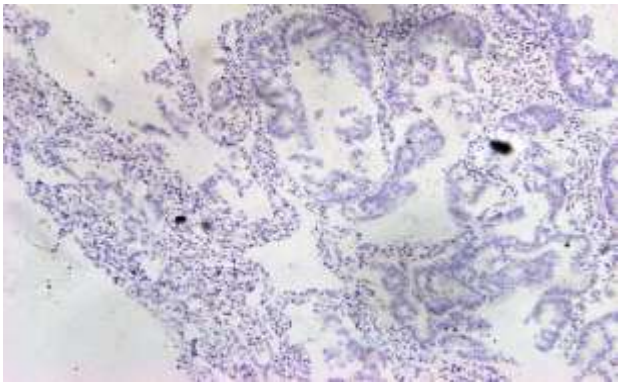

Figure 7: Ovarian mucinous carcinoma (case no-25, WT1 stain ×400).

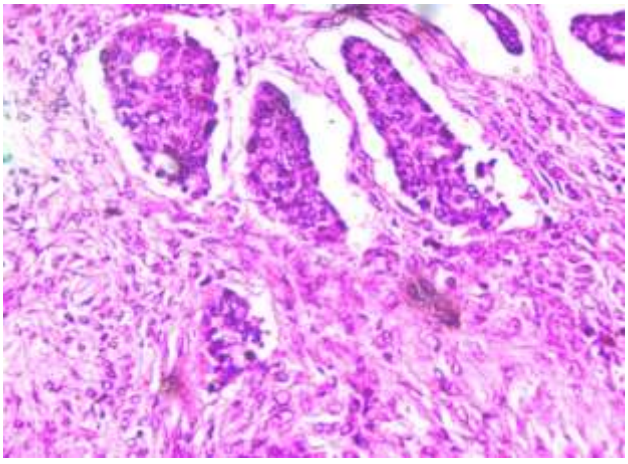

Figure 8: Ovarian endometrioid carcinoma (case no-20, H & E stain ×400).

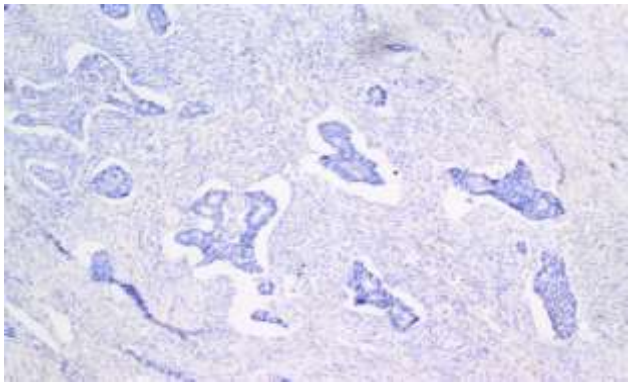

Figure 9: Ovarian endometrioid carcinoma (case no-20, WT1 stain ×100).

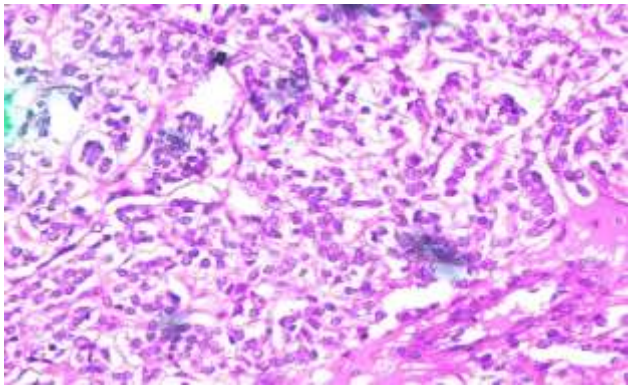

Figure 10: Clear cell carcinoma (case no-29, H & E stain ×400).

The (Table 4) showed WT1 Expression in different histologic subtypes of ovarian carcinoma. According to the histologic subtypes, WT1 was mainly expressed in serous

carcinoma. All the serous carcinoma (16/16) showed a positive reaction for WT1. Among them, 14 cases (87.5%) showed >50% (3+) WT1 score and two (2) cases (12.5%) showed 11% to 50 % (2+) scores. Regarding endometrioid carcinoma four (4) (66.6%) of six (6) were negative whereas one (1) (16.6%) showed 1% - 10% (1+) and one (1) (16.6%) showed 11 % - 50% (2+) WT1 scores. All the mucinous (4/4) and all the clear cell carcinoma (3/3) were negative with less than 1 % of the tumor cells stained two (2) cases of undifferentiated carcinoma were also negative. There was a significant relation between WT1 expression and different histologic subtypes of epithelial ovarian carcinoma with a p value of 0.001.

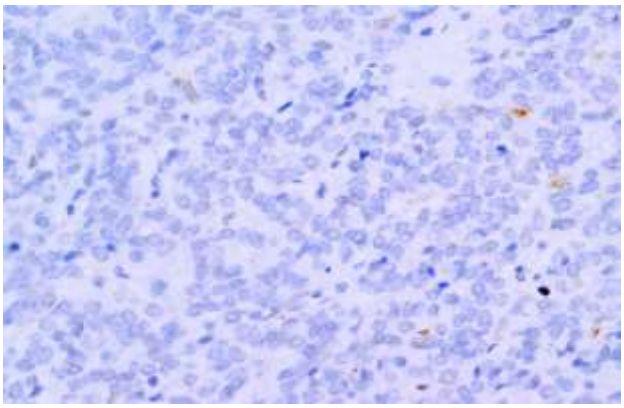

Figure 11: Clear cell carcinoma (case no-27, WT1 stain ×400).

DISCUSSION

In this cross-sectional type of descriptive study, a total number of 31 cases were examined at the Department of Pathology, RMC and Department of Pathology, BSMMU, from July 2019 to June 2021. Both H & E and WT1 staining were done successfully in 31 cases and were available for interpretation. Among 31 cases of epithelial ovarian carcinoma 16 cases were serous carcinoma, six (6) cases were endometrioid carcinoma, four (4) cases were mucinous carcinoma, three (3) cases were clear cell carcinoma and the remaining two (2) cases were undifferentiated types. Clinically suspected but not histopathologically confirmed cases and ovarian tumors other than epithelial types were excluded from the study. The age of the study sample ranged from 20-75 years in the present study. Among the 31 cases of ovarian epithelial carcinoma median age of the cases was 47 years. Most of the cases (29%) were in the age group of 51-60 years. The next frequent age group was 31-40 (25.8%). This result is comparable with the studies done in Iran by Arab et al and in Singapsore by Hwang et al.[12,13] While this result is not comparable with some previous studies done by SEER, USA, 1992-1999 where the median age is much higher, 60 and 63 years respectively. This may be due to some ethnic variability which needs further studies.[14] The present study showed no significant correlation between patient age and degree of WT1 expression (p>0.05). This result is comparable with Hafedh et al and Tanaka et al which revealed no significant relationship between age and the expression of WT1.[15,16] In the present study, serous carcinomas showed the most frequent immunoreactivity for WT1 among ovarian carcinomas. All the serous carcinomas included in our study were positive. The result is comparable to that reported by Shimizu et al.[17] Since 87.5% of the serous carcinomas had a strong positive reaction for WT1 in more than 50% of the tumor cells, the sensitivity in the present study is higher than in the previous studies of Hashi et al and Goldstein et al which had a corresponding rate of diffuse reaction in 72% and 73% of their cases, respectively.[18,11] Although 100% of the serous carcinomas in our study were positive, other studies by Goldstein et al and Lee et al indicate that a WT1 negative reaction does not exclude the diagnosis of a serous ovarian carcinoma.[10,11] In the present study all the mucinous and clear cell carcinomas were negative. This finding correlates with the results reported by Goldstein et al and Hashi et al. In contrast, Shimizu et al found some immunohistochemically expression of WT1 in both mucinous and clear cell carcinomas. They found that serous carcinomas had a significantly higher expression than clear cell carcinomas but not a significantly higher expression than mucinous carcinomas. Differences in the results may be due to differences in the immunohistochemical protocols, differences in sample size, and the use of different primary antibodies.[11,17,18] The endometrioid carcinomas included in the present study showed either no reaction or a diffuse reaction, with 1-10% and 11-50% of the tumor cells being positive for WT1. Shimizu et al found a low degree of WT1 expression among endometrioid carcinomas, and the expression was significantly less than among serous carcinomas.[17] Lee et al and Logani et al found WT1 expression in some of the endometrioid subtypes, but only a very limited number of endometrioid carcinomas were included in their studies.[10,19] The differences in WT1 expression of the serous subtypes compared with the clear cell and the mucinous cell type support other studies that showed the immunohistochemical expression of WT1 seems to reflect biological cell type rather than mutations.[20] As suggested by Gilks subtypes of epithelial ovarian carcinoma differ from each other in gene expression profile and also in biological behavior. This theory may explain the different expressions of WT1.[21] Hundred percent of the undifferentiated ovarian carcinomas included in the present study showed no WT1 expression, whereas in the study of Waldstrom et al 80% of undifferentiated carcinoma showed no WT1 expression whereas the remaining 20% were diffusely positive. The result might reflect that some of the histologic undifferentiated carcinomas are very low differentiated serous (or endometrioid) carcinomas, whereas the WT1 negative carcinomas may be of other biological cell types.[22] The present study demonstrates differences in immunohistochemical expression of WT1 among different histologic subtypes of ovarian carcinomas, supporting previous studies that identified serous carcinoma as the most frequent type expressing WT1.

Limitations

The study was conducted in a single center with a small sample size. So, the results may not represent the whole community.

CONCLUSION

In the present study, there was a significant relation between WT1 expression and different histologic subtypes of ovarian carcinoma ($p<0.05$). So, WT1 can be applied by both the histopathologist and clinician as an effective marker regarding diagnosis and patient management. It can be concluded that the expression of WT1 in different histologic subtypes of ovarian carcinoma can be applied to epithelial ovarian carcinomas in positive and differential diagnoses.

Recommendations

Additional immune markers like PAX8 may be used with WT1 for a more effective diagnosis of epithelial ovarian carcinoma.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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