Research Article

Immunohistochemical Expression of Flotillin1 in Ovarian Serous Carcinoma

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Abstract
Background: Ovarian cancer is one of the most common cancers among females worldwide. Its pathogenesis is not well understood till now. FLOT-1 is expressed in a wide range of normal and neoplastic tissues and has emerged as an important regulator cellular adhesion, proliferation, and metastasis. Its role in ovarian serous carcinogenesis is not clear and needs further studies. **Methods:** The present study comprised 72 cases of serous ovarian tumors including; 50 (69.44%) cases of serous ovarian carcinoma, 12 (16.67%) cases of borderline serous ovarian tumors and 10 (13.89%) cases of serous ovarian cyst-adenoma. Immunohistochemistry for FLOT-1 antibody was performed using the avidin biotin-peroxidase complex method. **Results:** FLOT-1 was positive in 54% of serous ovarian carcinoma cases, 50% of borderline cases were positive and all serous cystadenoma cases were negative. **S**tatistically significant association was detected between FLOT-1 expression and grade of differentiation of serous ovarian carcinoma cases (p=0.000). Meanwhile, no statistically significant association was found between FLOT-1 expression and age, tumor size, tumor bi-laterality and tumor stage (p=0.179, 0.643, 0.093, 0.187 respectively). In serous borderline cases, there were no statistically significant association between FLOT-1 expression and age, tumor size and tumor bi-laterality (p= 0.558, 0.558, 0.121 respectively). **Conclusions:** FLOT-1 expression is significantly associated with poor prognostic factors and thus can be used as a prognostic indicator for serous ovarian carcinoma patients.
Key words: FLOT-1; Serous Ovarian Neoplasms, Immunohistochemistry.
All authors have no conflict of interest.

Introduction
Ovarian cancer is one of the most common causes of cancer related-deaths in females. It is the 8th most common cancer among women in the world and considered the 5th cause of cancer related death in women with a higher mortality rate than all cancers of female genital tract. It is associated with the worst prognosis (Siegel et al., 2015; Ferlay et al., 2015; Cabasag et al., 2020).

In Egypt it has been reported that ovarian cancer cases comprise 4.5% of all registered cancer cases. It is the fourth most common cancer among females (Ibrahim et al., 2014).

In developed countries, 90% of malignant ovarian neoplasms are epithelial, 5%–6% of tumors originate from sex cord-stromal cells, and 2%–3% of the neoplasms arise from germ cells (Younes and Zayed, 2019).

According to the last registries of NCI in Egypt, surface epithelial malignant tumors accounts for 73.33% of all types of malignant ovarian tumors. Serous malignant tumors represent 34.82% between surface epithelial malignancies (Mokhtar et al., 2007, Nassar et al., 2015).

Flotillins are also known as Reggies; reggie-1 points to Flotillin2 (FLOT-2), while reggie-2 points to Flotillin1 (FLOT-1) (Banning et al., 2014). Flotillins are known to be lipid rafts scaffolding proteins which are cell membrane specialized domains. They act as physical platforms for variable molecules to organize multiple signal transduction processes (Staubach and Hanisch, 2011).

Flotillins are abundantly expressed in all mammals but they are also present in bacteria, fungi, plants and metazoans. FLOT-1 appears to be more restricted in mammalian. FLOT-1 is expressed mostly in brain, heart, placenta, lung and in hematopoietic cell (Banning et al., 2014). FLOT-1 in humans is present on chromosome 6p21.3 (Zhao et al., 2011). It is related to cell signal transduction, intercellular adhesion and endocytosis (Banning et al., 2011). It has been shown that FLOT-1 overexpression & up-regulation also occurs in different types of human cancers and was correlated with their development and progression of cervical cancer and breast cancer (Yan et al., 2014; Zhang et al., 2014; Butz et al., 2015).

Regarding ovarian cancer, the exact association between FLOT-1 and ovarian cancer develop-ment is still unclear, how-ever, a single study was done and revealed that FLOT-1 protein expression was significantly related to serous tumors (Li et al., 2018).

Material and Methods

Tissue specimens

The present study comprised 72 randomly selected cases of serous ovarian tumors divided into 50 cases of serous ovarian carcinoma, 12 cases of borderline serous tumors and 10 cases of benign serous cyst-adenoma. Serous ovarian carcinoma cases grade were evaluated by three-tier grading system (Murakami et al., 2015). Tumor stage was estimated by FIGO staging system (Prat, 2014).

Immunohistochemistry

Primary antibody against FLOT-1: (Poly-clonal rabbit antibody 100 µl, concentrated, Clinilab Laboratories, USA). Each run contained a Positive control tissue section and was processed in the same manner as the patient tissue samples. The positive control was normal gastric mucosa.

Scoring of Immunostaining

Immunohistochemical staining analysis was performed without previous review of patient clinical data. We noticed that FLOT-1 is expressed in both cell membrane and cytoplasm. The proportion of positive cells was scored as follows: 0= no positive cells; 1, ≤25% positive cells; 2, 26–50% positive cells; 3, 51–75% positive cells and 4 = >75% positive cells. 0 = no staining; 1 = weak staining; 2, moderate staining and 3 for strong staining. A final immune-reactivity score, or staining index (SI), was made by the sum of the positive proportion and staining intensity scores, with 0 as ≤2 points; 1 as 3–4 points; 2 as 5–6 points; and 3 as 7 points. SI scores of 0 and 1 were FLOT1 negative; scores of 2 and 3 were FLOT1 positive (Li et al., 2018).

Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS software version 16).

Results

Clinicopathological Features

Table (1): Clinico-pathological features for patients with serous ovarian carcinoma (n=50).

Clinico-pathological features	No. (%)
Age	
<50	21 (42%)
≥50	29 (58%)
Tumor size	
<10	20 (40%)
≥10	30 (60%)
Tumor grade	
Grade II	31 (62%)
Grade III	19 (38%)
FIGO stage	
I	30 (60%)
II	13 (26%)
III	7 (14%)
Tumor bi-laterality	
Unilateral	24 (48%)
Bilateral	26 (52%)

Table (2): Clinico-pathological features for patients with serous ovarian borderline tumors (n=12).

Clinico-pathological features	No. (%)
Age	
<28	5 (41.7%)
≥28	7 (58.3%)
Tumor size	
<8.5	7 (58.3%)
≥8.5	5 (41.7%)
Tumor bi-laterality	
Unilateral	10 (83.33%)
Bilateral	2 (16.67%)

Table (3): Clinico-pathological features for patients with serous ovarian cyst-adenoma (n=10)

Clinico-pathological features	No. (%)
Age	
<51	5 (50%)
≥51	5 (50%)
Tumor size	
<2.5	5 (50%)
≥2.5	5 (50%)
Tumor bi-laterality	
Unilateral	8 (80%)
Bilateral	2 (20%)

Immunohistochemical expression of FLOT-1

Table (4): FLOT-1 immunohistochemical expression in different types of serous ovarian tumors.

	Negative	**Positive**
Benign (n=10)	10 (100%)	0 (0%)
Borderline (n=12)	6 (50%)	6 (50%)
Malignant (n=50)	23 (46%)	27 (54%)

Table (5): Association between FLOT-1 expression and clinic-pathological features for patients with serous ovarian carcinoma (n=50)

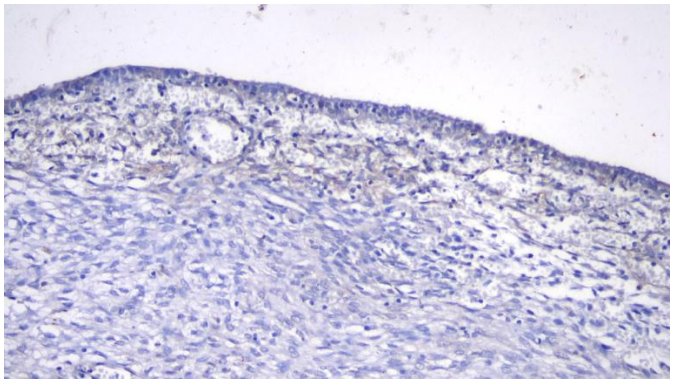
Clinico-pathological features	**Total 50 (100%)**	**FLOT-1 expression SI**		**P value**
		(Negative) (n=23)	**(Positive) (n=27)**	
Age				
<50	21 (42%)	12 (57.14%)	9 (42.86%)	0.179
≥50	29 (58%)	11 (37.93%)	18 (62.07%)	
Tumor size				
<10	20 (40%)	10 (50%)	10 (50%)	0.643
≥10	30 (60%)	13 (43.33%)	17 (56.66%)	
Tumor grade				
Grade II	31 (62%)	23 (74.19%)	8 (25.8%)	0.000***
Grade III	19 (38%)	0 (0%)	19 (100%)	
FIGO stage				
I	30 (60%)	15 (50%)	15 (50%)	
II	13 (26%)	7 (53.84%)	6 (46.15%)	0.187
III	7 (14%)	1 (14.29%)	6 (85.71%)	
Tumor bi-laterality				
unilateral	24 (48%)	14 (58.33%)	10 (41.66%)	0.093
bilateral	26 (52%)	9 (34.61%)	17 (65.38%)	

*** P - value <0.05 are considered statistically significant according to Chi-Square test and Fisher's exact test.**

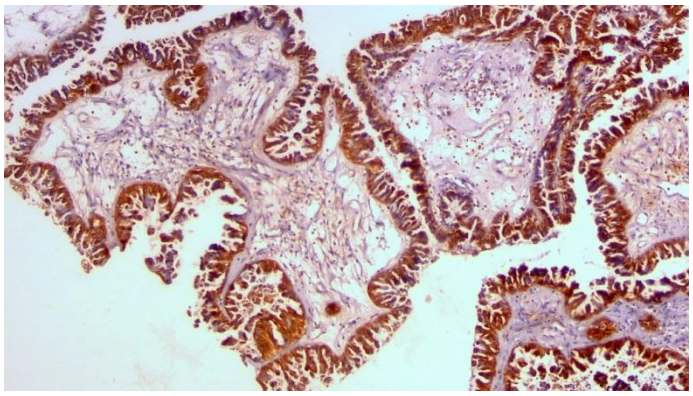
Table (6): Association between FLOT-1 expression and clinic-pathological features for patients with serous ovarian borderline tumors (n=12).

Clinico-pathological features	**Total 12 (100%)**	**FLOT-1 expression SI**		**P value**
		Negative) (n=6)	**(Positive) (n=6)**	
Age				
<28	5 (41.7%)	3 (60%)	2 (40%)	0.558
≥28	7 (58.3%)	3 (42.86%)	4 (57.14%)	
Tumor size				
<8.5	7 (58.3%)	4 (57.14%)	3 (42.86%)	0.558
≥8.5	5 (41.7%)	2 (40%)	3 (60%)	
Tumor bi-laterality				
unilateral	10 (83.33%)	4 (40%)	6 (60%)	0.121
bilateral	2 (16.66%)	2 (100%)	0 (0%)	

*** P - value <0.05 are considered statistically significant according to Chi-Square test and Fisher's exact test.**



Fig(1): Negative FLOT-1 expression in the lining of serous ovarian cyst-adenoma (streptavidin-biotin-immunoperoxidase X 200)



Fig(2): Strong positive FLOT-1 expression in serous ovarian borderline tumor (streptavidin-biotin-immunoperoxidase X 100).

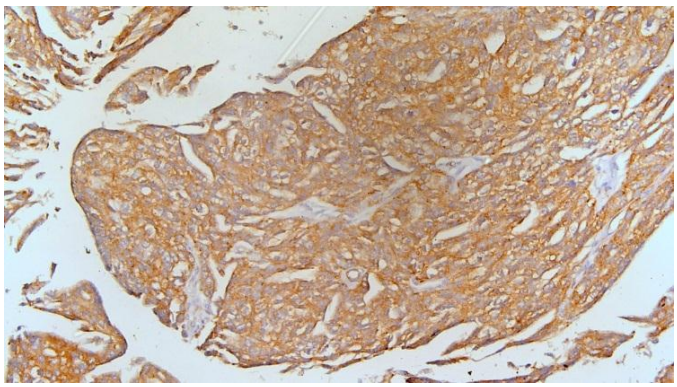


Fig (3): Strong positive FLOT-1 expression in serous ovarian carcinoma grade III (streptavidin-biotin-immunoperoxidase X 200).

Discussion

In the current study, 54% of serous carcinoma cases were considered positive. This was not in line with Li et al., (2018) who reported that 91.89 % of malignant cases were positive. In borderline tumors, FLOT-1 expression was positive in 50% of cases. A finding which was not in accordance with Li et al., (2018), who detected positivity in 75% of borderline cases. These differences could be due to the difference in sample size. Their study included 37 malignant cases, 4 borderline cases and 3 benign cases only. Meanwhile, our study included 50 malignant cases, 12 borderline cases and 10 benign cases. It might be also due to the usage of different FLOT-1 antibody as ours was polyclonal while it was monoclonal in their study. Among cyst- denoma cases, all cases were negative for FLOT-1.

These results agreed with Li et al., (2018) who found 100% of benign cases negative also. Regarding age, in the present study, there was no statistically significant association between FLOT-1 expression in serous carcinoma cases and age (p= 0.179). This was in line with Li et al., (2018) who reported a statistically non-significant association between FLOT-1 expression and age (p= 0.660). This was also agreed with Koh et al., (2016) who reported a statistical non-significant association between FLOT-1 expression and age with (p= 0.214) during their study on role of FLOT-1 in breast cancer.

No studies have reported a statistical significance between FLOT-1 expression and age in any type of tumors.

Regarding tumor size, we reported a statistically non-significant association between FLOT-1 expression and tumor size (p=0.643). This agreed with Li et al., (2016) who reported a statistically non-significant association between FLOT-1 expression and tumor size (p=0.427) during their study on FLOT-1 expression in prognosis of cervical cancer. This was not in accordance with Zhang et al., (2013) who reported that there was a statistically significant association between FLOT-1 expression in hepatocellular carcinoma with tumor size with (p =025).

This difference might be due to the usage of a scoring system different from ours. Concerning bi-laterality, we reported non-significant statistical association between FLOT-1 expression and bi-laterality with (p =0.093). To the best of our knowledge, no previous studies did examine the association between FLOT-1 expression and bi-laterality in any type of bilateral tumors. Regarding tumor stage, we reported a statistically non-significant association between FLOT-1 expression and stage of tumor (p =0.187). This was similar to the results reported by Li et al., (2018) who found a statistically non-significant association between FLOT-1 expression and tumor stage (p=0.609). Zhang et al., (2014) reported a statistically significant association between FLOT-1 expression and tumor stage also (p <0.05) in their study on renal cell carcinoma.

We reported a statistically significant association (p=0.000) between FLOT-1 expression and tumor grade. This was in accordance with Zhang et al., (2014) who reported a statistically significant association between FLOT-1 expression and tumor grade in renal cell carcinoma (p <0.05). This also was in line with Baig et al., (2019) who reported a statistically significant association between FLOT-1 expression and tumor grade (p=0.023) in colon cancer. On the other hand, Li et al., (2018), reported no statistically significant association with grade of tumour differentiation with (p=0.160) in their study on FLOT-1 expression in ovarian epithelial tumors and that was not in correspondence to our study. This might be due to difference in sample size.

In this study we found that there was no statistical significance in borderline tumors between FLOT- 1expression between age,tumor size and bi-laterality (p=0.558, 0.558, 0.121 respectively). This was in line with Li et al., (2018) who reported a statistically non-significant association between FLOT-1 expression in serous borderline tumors with age (p=0.660). In this study, all benign cases were negative for FLOT-1. We noticed that Li et al., (2018) reported that there was no statistical significance between FLOT-1 expression with age, tumour size and bi-laterality in all benign

ovarian tumors including serous, mucinous and transitional subtypes.

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