

Overexpression Of B7-H4 And Its Co-Relation With Biological Behavior In Ovarian Tumors

Marvi Raza¹, Fouzia Shaikh², Nasima Iqbal³, Fauzia Perveen⁴, Ghazala Masood Farrukh⁵, Hira Naeem⁶

¹Lecturer, Dept. of Pathology, Ziauddin University, Clifton Campus, Karachi, Pakistan

²Professor, Head of Department, Dept. of Pathology, Ziauddin University, Clifton Campus, Karachi, Pakistan

³Associate Professor, Dept. of Pathology, Baqai Medical College, Baqai Medical University, Karachi, Pakistan.

⁴Assistant Professor, Dept. of Biochemistry, Liaquat College of Medicine and Dentistry, Karachi, Pakistan

⁵Assistant Professor, Dept. of Physiology, Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan

⁶Assistant Professor, Dept. of Pharmacology, Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan

Address for correspondence:

Dr. Marvi Raza

Lecturer, Dept. of Pathology, Ziauddin University, Clifton Campus, Karachi, Pakistan Email: drmarvi.shaikh@gmail.com

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Abstract

Background

Epithelial ovarian cancer is the eighth most common malignancy in women, with the highest mortality rates regardless of significant advances in therapeutic techniques. This is due to the fact that the symptoms of epithelial ovarian tumors are vague and nonspecific, concealing the disease's early onset and progression. Several immunological markers, including CA125, AFP, Inhibin, and B7-H4, have been identified to aid in the diagnosis of epithelial ovarian tumors. The expression of the novel biomarker B7-H4 in epithelial ovarian tumors tissues is investigated in this study. We also investigated the link between B7-H4 expression and biological behaviour as well as risk factors.

Methodology

This cross-sectional study validated the diagnosis of epithelial ovarian tumours and histological characterization by examining morphological features. B7-H4 expression was examined immunohistochemically in the most representative tumour block. Mean frequency and percentages were calculated for quantitative variables. The chi-square test and Fisher's Exact Test were used to examine qualitative variables, and P values less than 0.05 were considered significant.

Results

IHC expression was found to be positive in 50 of 66 cases (75.7%). The majority of patients with B7-H4 IHC positivity have benign epithelial ovarian tumours (25/50; 50%), followed by malignant (19/50; 38%) and borderline (6/50; 12.5%) tumours. This demonstrates a statistically significant ($p=0.004$) link between B7-H4 and epithelial ovarian tumours. Furthermore, epithelial ovarian tumours with distinct morphology, such as Brenner tumours of the ovary, were discovered to express the B7-H4 protein 2/2. Statistics show a link between B7-H4 protein expression and these unusual mutations. On a scale of one to four, the majority of the positive samples had a protein expression strength of one, with a statistically significant estimate.

Conclusion

B7-H4 expression has been linked to epithelial ovarian tumours, and it is expected to emerge as a new molecular marker to assess the biological activity of these tumours. Other members of the B7 family, such as B7-H3, B7-H5, B7-H6, and B7-H7, may be able to shed more light on tumour immune evasion and aid researchers in the identification of new immunotherapeutic targets for the treatment of ovarian and other cancers.

INTRODUCTION

One of the most prevalent gynecologic cancers, following uterine and cervical cancer, is the ovarian cancer (1) (2). Additionally, Epithelial ovarian tumor has the worst prognosis and the highest fatality rate (3). According to predictions, the death rate of this malignancy will considerably increase by the year 2040 (3). The majority of ovarian tumors (65%) are surface epithelial neoplasms, which are the most common type. (4) Epithelial cancer is three times more deadly than breast cancer, although having a lower frequency. This is because the disease is harder to detect early and there are less effective treatment choices available(5). Only 46% of women with epithelial ovarian neoplasm survive past the age of five because of nonspecific symptoms, inadequate screening tools, late identification, and treatment resistance there is no anatomical barrier to stop tumor spreading from ovaries into the peritoneal cavity, abdominal, and pelvic organs (6) (7). Epithelial ovarian cancer has been identified in 295,414 cases, according to a World Cancer Research Fund estimate from 2018. By 2040, 434,184 cases of ovarian cancer were estimated, having incidence and mortality rates that are age-standardized of 6.6 and 3.9, respectively.(8) It is the second most frequent type of cancer-related death after breast cancer in Pakistani women. There are 4.8% epithelial ovarian cancers per 100,000 in Pakistani women each year, compared to 7.2 in India (9). In Karachi, there are 10.2 epithelial ovarian cancers per 100,000 people per year (10).

Epithelial ovarian carcinomas are diagnosed in the great majority of postmenopausal women, with a median age of 63 years (11). Although the cause of epithelial ovarian tumor is unknown, hormonal, environmental, and genetic variables all have a part in its progression (12). A high-fat diet, talc or asbestos exposure, early menarche, late menopause, null parity, age, and ethnicity have all been associated with the risk of ovarian cancer (13, 14).

B7-H4 is a member of the B7 family that is well known for playing a significant part in the down regulation of the immune response to an antigen (also known as B7x or B7S1). B7-H4 mRNA is broadly distributed, although its expression in tissues appears to be limited. B7-H4 is induced by a variety of cytokines and inflammatory mediators. Recently, B7-H4 has been demonstrated to suppress T-cell activity by impairing T-cell expansion, cell cycle arrest, cytokine production, and inducing apoptosis in activated T-cells. A growing body of research also demonstrates that B7-H4 expression levels correlate with cancer size, histological type, pathologic stage, grade, infiltration, lymph node metastasis, cancer progression, recurrence, and death in a range of malignancies (15)

The goal of current study is to analyze the B7-H4 protein expression on biological behavior of epithelial ovarian tumors.

MATERIALS AND METHODS

Histological Diagnosis and Tissue Sampling

In this trial, we collected 66 formalin-fixed, paraffin-embedded tissue blocks of epithelial ovarian tumors from Ziauddin University and Hospital North campus in Karachi. There were 23 cases of serous cystadenomas, 2 cases of serous tumors with low malignant potential, 15 cases of serous cystadenocarcinomas, 12 cases of mucinous cystadenomas, 4 cases of mucinous borderline tumors, malignant mucinous cystadenocarcinomas were 6 cases and epithelial benner tumors 2 cases. Prior to surgery, Radiotherapy and chemotherapy were not used on any of the patients. The American Joint Committee on Cancer's (AJCC) tumor-node-metastasis (TNM) staging method was used after looking through the pathology findings. The clinical characteristics of the patients are shown in Table 1. In addition, healthy tissues from the gallbladder's non-cancerous portion were extracted and used as controls. The ethics committee of Dr Ziauddin University provided ethical approval for this research project, and approval was obtained in compliance with institutional norms. (Ref # 1721219MRPAT).

Immunohistochemistry

For immunohistochemistry, the block with the greatest tumor volume was chosen as the most representative. This method was used in this work by Signoretti et al. The most representative block was determined to be the one with the greatest tumor volume. In a nutshell, 3mm-thick tissue blocks embedded in formalin-fixed, paraffin wax were sliced, then placed on glass slides after being soaked in hot water. After that, antigens were removed in a microwave for 15 minutes with 0.1mol/L citrate buffer. At a dilution of 1:50ml, a monoclonal mouse antiB7-H4 antibody was used. A buffer washing was carried out after the preparation had been applied to the tissue section for 45 minutes prior to the development of the enzyme activity separately. After cleaning, the slices underwent a one-minute hematoxylin counterstain than dried at room temperature, and dehydrated. The coverslip was then adhered to the mounting media with adhesive. Gallbladder slides that were B7-H4 positive served as positive controls, whereas tissue slides devoid of primary antibodies served as negative controls. The B7-H4 staining pattern was evaluated using IHC sections by the same skilled histopathologists. The presence of dark brown cytoplasmic stains in tissue slices was considered positive under light microscopy.(16)

Evaluation of Immunohistochemical Staining of B7-H4

B7-H4 expression was considered positive in every instance that displayed at least a little staining. After carefully analyzing the entire histologic slice, the percentage of B7-H4 positive cells was determined, and a final score was provided (1+ 0–10% positive cells, 2+ >10–50% positive cells, 3+ >50–80% positive cells, and 4+ >80–100% positive cells).Each instance that showed at least slight focused staining had B7-H4 expression. The percentage of B7-H4 positive cells was calculated after carefully evaluating the entire histologic slice, and a final score was assigned (1+ 0–10% positive cells, 2+ >10–50% positive cells, 3+ >50–80% positive cells, and 4+ >80–100% positive cells). (17)

Statistical Analysis

SPSS Statistics 21 was used to conduct the statistical analysis. For qualitative data, frequency and percentages were employed, whereas mean and standard deviation were used for quantitative variables. The Chi-square test was used to examine the B7-H4 expression in the biological behaviors of the tumor, such as benign, malignant, and borderline epithelial ovarian cancers. It was determined that a p-value of 0.05 was statistically significant.

RESULTS

The current study involved 66 participants. There were six borderline tumours (2 serous borderline and four mucinous borderline tumours) and 21 malignant epithelial ovarian tumours. There were 15 cases of serous cyst adenocarcinoma and 6 cases of mucinous cyst adenocarcinoma, and 39 cases were benign (23 serous cyst adenoma, 12 mucinous cyst adenoma, 2 Seromucinous cyst adenoma and 2 benign Brenner tumor) as shown in **Table I**.

Table I: Demographic and Clinicopathological Characteristics of All Cases

Demographic and Clinicopathological Characteristics	No of Cases (n=66)	Frequency (%)
TUMOR		
➤ Benign	39	59.09
➤ Borderline	06	9.09
➤ Malignant	21	31.8

VARIANTS OF EPITHELIAL OVARIAN TUMORS		
➤ Serous adenocarcinoma	15	22.7
➤ Serous borderline tumor	02	3.03
➤ Serous cystadenoma	23	34.8
➤ Mucinous adenocarcinoma	06	9.09
➤ Mucinous borderline tumor	04	6.06
➤ Mucinous cystadenoma	12	18.1
➤ Brenner tumor	2	3.0
➤ Seromucinous tumor	2	3.0
AGE		
➤ ≤ 50	39	59.09
➤ >50	27	40.90
MARITAL STATUS		
➤ Single	11	16.6
➤ Married	55	83.3
• Nulliparous	12	21.8
• Multiparous	43	78.2
SIDE		
➤ Right	21	31.8
➤ Left	46	69.6
GRADE		
➤ Well Differentiated	07	9.8
➤ Moderately Differentiated	03	4.2
➤ Poorly Differentiated	11	15.4
STAGE		
➤ I A	09	12.6
➤ I C	02	2.8
➤ II A	08	11.2
➤ III C	02	2.8

B7-H4 immunohistochemistry expression was found to be positive in 40 of 66 epithelial ovarian cancers (60.6%). B7-H4 immunohistochemical analysis of 40 positive cases revealed that the majority (20/40) were benign, 17/40 were malignant, and 6/40 were borderline. As shown in Table II, there was a statistically significant link between B7-H4 and ovarian epithelial malignancies ($p= 0.004$).

Table II: Clinicopathological and Demographic Characteristics of Ovarian Tumors and Statistical Estimates with B7-H4

Demographic and clinicopathological characters	n=66	B7-H4		P-value
		Positive	Negative	

TUMOR				
➤ Benign	39	20	19	0.004
➤ Borderline	06	06	00	
➤ Malignant	21	17	04	
MARITAL STATUS				
➤ Single	16	11	05	1.00
➤ Married	55	37	18	0.801
• Nulliparous	12		09 03	
• Multiparous	43		28 15	
SIDE				
➤ Right	25	18	07	0.606
➤ Left	46	30	16	
AGE				
➤ ≤ 50	48	34	14	0.427
➤ > 50	23	14	09	
GRADE				
➤ Well Differentiated	07	06	01	0.427
➤ Moderately Differentiated	03	02	01	
➤ Poorly Differentiated	11	09	02	
STAGE				
➤ I A	09		09 00	0.152
➤ I C	02		01 01	
➤ II A	08		06 02	
➤ III C	02		01 01	

27/40 serous epithelial ovarian neoplasms, with 13/40 serous cystadenocarcinomas, 2/40 low-risk serous tumours, and 12/40 serous cystadenomas, were shown to express the B7-H4 protein. Mucinous cystic tumours are the second most frequent morphological form, and 14/22 tumour tissues strongly express the B7-H4 protein, with 4/22 of these tumours being mucinous cystadenocarcinomas and 4/22 being mucinous borderline cystic tumors and 6/22 are mucinous cyst adenomas. With p values of 0.051 and 0.195, they show an insignificant statistically relationship with B7-H4 expression. B7-H4 protein expression was also discovered in some uncommon morphological subtypes of Brenner tumors, which are epithelial ovarian cancers. Additionally, these rare variants significantly correlate with the expression of the B7-H4 protein (p-Value: 0.011) as shown in **Table III**. Protein expression strength in the majority of positive samples was 1 on a scale of 4, with a statistically significant estimate of p-value 0.025 was observed with serous ovarian tumor as shown in **Table IV**.

Table III: Association of B7-H4 Expression with Morphological Variants of Ovarian Tumors

Histological variants	B7-H4 Positive	B7-H4 Negative	P- Value
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Serous Tumors	Serous adenocarcinoma n=15	13	02	0.051
	Serous borderline tumor n=02	02	0	
	Serous cyst adenoma n=2	12	11	
Mucinous Tumors	Mucinous adenocarcinoma n=06	04	02	0.195
	Mucinous borderline tumor n=04	04	0	
	Mucinous cystadenoma n=12	06	06	
Other Rare Variants				0.011
	Brenner tumors n=02	02	0	
	Seromucinous cyst adenoma n=02	0	02	

Table IV: Immunohistochemical Staining Intensity of B7-H4 in Epithelial Ovarian Tumors with Statistical Estimates

Positive Histological Variant	Number of cases	IHC Scoring				P-value
		1+	2+	3+	4+	
SEROUS OVARIAN TUMORS						0.0225
• Serous adenocarcinoma	13	01	06	05	01	
• Serous borderline tumor	02	02	00	00	00	
• Serous cyst adenoma	12	08	04	00	00	
MUCINOUS OVARIAN TUMORS						0.440
• Mucinous adenocarcinoma	04	02	02	00	00	0.290
• Mucinous borderline tumor	04	03	00	01	00	
• Mucinous cystadenoma	06	02	03	01	00	

OTHERS						
• Brenner tumors	02	00	00	02	00	

DISCUSSION

According to WHO classification, surface epithelial ovarian tumors are the most prevalent type of tumor found worldwide. With five-year survival rates of fewer than 45 percent, epithelial ovarian cancer is the eighth most common cancer among women globally. Age-standardized rates are rising in many low- and middle-income countries whereas they are dropping in the majority of high-income countries.(18) Additionally, as individuals live longer, there are more cases being diagnosed year. In order to control epithelial ovarian cancer, we must comprehend its causes. This will improve the ability to identify those who are most at risk and may require screening, while identifying potential modifiable reasons may enable intervention to cut rates. (19) Recent studies have identified several markers on the surface of tumor stem cells. B7-H4, a new member of the B7 family which acts by decreasing T-cell immunologic responses by preventing the production of cytokines and the advancement of the cell cycle. Despite being present in a variety of tumor types, in healthy tissues, B7-H4 protein expression is not frequently found. Numerous tumors, including lung tumors, cervical cancer, and other epithelial malignancies, exhibit high levels of B7-H4 expression that have been associated with tumor genesis, progression, and prognosis.(20) Future studies may concentrate on B7-H4 overexpression in tumor tissues as a potential target for tumor diagnosis or treatment.

The levels of B7-H4 expression in 66 patients with epithelial ovarian tumors were examined in the current study. We discovered that out of 66 epithelial ovarian tumor biopsies, 39 (59.09%) were benign, 6 (9%) were borderline, and 21 (31.8%) were malignant. Histological heterogeneity is seen in epithelial ovarian tumors. In contrast to the variety in the expression of B7-H4 in different cases, on tumor cells, B7-H4 was primarily expressed. What we discovered was the poorer histological differentiation, local invasion, a worse response to systemic chemotherapy, and a shorter survival time were all related with tumor cells that expressed more B7-H4. These results suggest that enhanced B7-H4 expression is related to tumorigenesis. During the cancer-immunity cycle, B7-H4 prevents the immune system from identifying and eliminating cancerous cells.(21)

Serous epithelial ovarian tumors, which have five primary histotypes and are the most prevalent pathologic subtype, mucinous epithelial ovarian tumors are the second most frequent epithelial ovarian tumor, each of which has its own genesis, etiology, molecular changes, risk factors, and prognosis. (22)

The B7-H4 protein was discovered to be extensively expressed in epithelial ovarian cancers in the current study, more frequently in serous tumors and mucinous tumors. Choi and colleagues utilized monoclonal antibodies comparable to those we used in our investigation and discovered that 85% (22/26) of epithelial ovarian tumors expressed B7-H4.(23) Using the monoclonal antibody, Tringler and colleagues discovered that 78% (18/23) of serous borderline tumors, 100% (32/32) of epithelial ovarian serous carcinomas, and 77% (20/26) of serous cystadenomas expressed B7-H4. (24) Another study by Barbara Tringler et al. reported that B7-H4 cytoplasmic and membranous expression was present in all primary serous (n = 32), endometrioid (n = 12), and clear cell carcinomas (n = 15), as well as all metastatic serous (n = 23) and endometrioid (n = 7) ovarian carcinomas. (24) These results are consistent with our findings and suggest that B7-H4 may play a part in the growth of ovarian tumors. It may also be a suitable marker for the identification of epithelial malignancies.

B7-H4 expression in epithelial ovarian tumor tissue was similar with expression in patients serum, according to research by Simon et al., indicating that B7-H4 expression in tissues might be an accurate representation of a patient's preoperative serum levels. (25) One of our drawbacks was that we were unable to compare the tissue and serum levels

While no additional variant was found in the current analysis, B7-H4 was reported to be strongly expressed in two Brenner cell malignancies. Contrarily, Tringler et al. discovered that all primary ovarian serous carcinomas (32 cases), endometrioid carcinomas (12 cases), clear cell carcinomas (15 cases), all metastatic serous carcinomas (23 cases), and all metastatic endometrioid carcinomas (7 cases) expressed 100% of B7-H4, but only 1 out of 11 cases with mucinous carcinomas. It is necessary to explain the disparity between their findings and those of this investigation in terms of the specificity of B7-H4 expression on a broader scale. Different risk exposure, genetic make-up, and cultural practices may be to blame for the distinctive uncommon variants identified in our area. (24) .

CONCLUSION

It was concluded that various tumor entities have distinctive and separate cellular origins because there is no significant statistical link between any clinicopathological features of ovarian tumor subtypes. As a result, they pursue independent and unique clinical courses. Alternatively, although a population-based database was employed, the sample sizes for several of the variations under study were rather small and most likely lacked sufficient statistical power.

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