Original Article

Study the Expression of Dickkopf-related Protein 3 Immunoreactivity in Serous Epithelial Ovarian Tumors

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Abstract

Background: Dickkopf-related protein 3 (DKK3) is regarded as a tumor suppressor in cancer tissue. *DKK3* gene effect has an important role in the prognosis of ovarian cancer with the possibility to discover new line treatment in ovarian cancer. Many cancers of the uterus, cervix, colon, rectum, and pancreas show an association between decreased DKK3 protein and poor outcomes in these cancers. **Objective:** The study aims to evaluate the immunoreactive scores of DKK3 in all serous epithelial ovarian tumors (serous cystadenoma, serous borderline tumor, and invasive serous adenocarcinoma). **Materials and Methods:** A cross-sectional research was evaluated by immunohistochemistry, and involved 47 cases of ovarian serous tumor. DKK3 immunoreactive scoring resulted from multiplying the positive cells (mean percentage) by the staining intensity of the ovarian tissue. Therefore, the DKK3 expression score involves a 0 (undetected), 1+ (weakly positive), 2+ (moderately positive), and 3+ (intensely positive). **Results:** Of 47 patients, 12 (25.5%) had benign serous cystadenoma, 14 had (29.8%) serous borderline tumor, and 21 (44.7) had invasive serous adenocarcinoma. DKK3 immunoreactive score was significantly high in different types of ovarian tumors. Tumor ovarian locations were highly significant, in which unilateral tumor ovarian locations were mostly presented in serous borderline tumors 92.9% (13 of 14), whereas bilateral locations of ovarian tumors were more in invasive serous adenocarcinoma 71.4% (15 of 21). There is a significant positive correlation between the immunoreactive DKK3 score and ovarian neoplasms in benign serous cystadenoma, as well as a positive correlation between immunoreactive DKK3 score and the location of ovarian neoplasms in both serous borderline tumor and invasive serous adenocarcinoma. **Conclusion:** Serous ovarian tumors have a significant decrease in DKK3 protein expression, especially in serous borderline tumors and invasive serous adenocarcinoma. The DKK3 protein expression is affected by age groups, site location of tumor, and not affected by tumor size.

Keywords: Dickkopf-related protein 3, Dickkopf Wnt signaling pathway inhibitor 3, immunohistochemistry, serous epithelial ovarian tumor

Introduction

Ovarian cancer is still a highly risky gynecological tumor in the world.[1,2] Ovarian cancer has progressive differentiation from high level (low grade) to poorly level (high grade), and a staging systematic begins from I to IV with spreading cancer to pelvic tissues (and formation of ascites)[3,4]

The initial stage of epithelial ovarian cancer (EOC) has relatively effective treatment, but a high percentage of ovarian cancers are diagnosed at stage III or IV[1,2] and a survival rate of 5 years is 30% classically.[5] Despite treatment of higher stages of EOC showing clinical improvement, it is associated with tumor relapse tumor.[1,6]

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A common EOC is serous subcategories (represented more than 70%) and associated with bad prognostic end stage in comparison to other subcategories of cancer (clear cell type, endometroid type, and mucinous type cancers).[1,6] Serous EOC is related to the epithelium of the surface of the ovary but some argue that serous cancer is related to epithelium of the fallopian.[5,7,8]

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Management therapy for EOC is surgery and chemotherapy,[9] and the effect of this chemical drug is estimated by serum tumor marker cancer antigen 125 and in addition to radiological investigation.[10,11]

The structure of the Dickkopf group contains four key proteins that include Dickkopf-related protein 1, Dickkopf-related protein 2, Dickkopf-related protein 3 (DKK3), and Dickkopf-related protein 4, which play roles in Wnt signaling pathway (antagonists function),[12] DKK3 function possibly will have task as stop proliferation (as tumor suppressor function) of cancer tissue by effect of Wnt signaling.[13]

Multiple research studies have shown that DKK3 may have a role as a tumor suppressor in various cancer types, due to its involvement in apoptosis.[14,15] In addition, this apoptosis process is done through the effects of P-glycoprotein, which may affect drug resistance and cell survival.[16] In addition, DKK3 is regarded as a tumor suppressor due to it is repeatedly omitted as associated with malignant tumor tissue.[17,18]

At the same time, the serum level of DKK3 is significantly reduced in ovarian cancer and gastrointestinal tract cancer (specifically gastric and colorectal) compared with the control group.[19-21] The *DKK3* gene effect may give true analysts for prognosis in ovarian cancer with the possibility of discovering new line treatment in ovarian cancer.[22] Many cancer tissues, such as the uterus, cervix, colon, rectum, and pancreas, show an association between decreased DKK3 protein and poor outcomes of cancer.[23-27] On the other hand, the medical importance of DKK3 in ovarian tumors (serous cancer type) and its treatment effect are not interpreted yet.[28]

The main objective of the existing research was to examine the expression of DKK3 protein immunohistochemistry in various types of serous epithelial ovarian tumors (serous cystadenoma, serous borderline tumor, and invasive serous adenocarcinoma) and their immunoreactivity scores in serous adenocarcinoma.

Materials and Methods

A retrospective research was conducted on 47 cases of ovarian serous tumors with a mean age and range of 44.276 and 17–78 years old, respectively. It was retrospective research as all patients who were admitted to the Marjan Medical City Hospital were surveyed about demographics and the investigation of data of ovarian serous tumors was between March 2022 and June 2023.

Paraffin blocks of ovarian serous neoplasm were prepared, and two slides were done for each type of neoplasm. The first slide is for establishing the diagnosis of ovarian serous neoplasm and the second slide is for antibodies DKK3 immunohistochemistry.

In calculating DKK3 immunoreactive expression, a scoring technique was resulted from multiplying positive cells (mean percentage) by the staining intensity of the ovarian tissue and so, the DKK3 expression score involves 0 (undetected), 1+ (weakly positive), 2+ (moderately positive), and 3+ (intensely positive).

The anti-DKK3 (AA 1-350) antibodies were obtained from antibodies-online GmbH, Schloss-Rahe-Str., 1552072 Aachen, Germany.

The cancer stage was evaluated using the International Federation of Gynecology and Obstetrics staging system, and the tumor grade and histological type were decided by the World Health Organization.[29]

The statistical analysis was performed using the Statistical Package for Social Sciences program (version 26, IBM Corp., Armonk, NY, USA). Resulting data were estimated as number (percentage), and mean ± standard deviation. Kruskal–Wallis test was used to association between DKK3 immunoreactive score and tumor size among benign serous cystadenoma, serous borderline tumor, and invasive serous adenocarcinoma. The chi-square test and one-way analysis of variance test were used to compare age groups and age mean correspondingly. A P value of <0.05 was considered statistically significant.

Ethical approval

The research was conducted following ethical guidelines. The study was conducted with the verbal and written consent of the patients before the study began. The research protocol, the patients, data, and the consent were accepted by the Babylon University/College of Dentistry a local ethics commission according to the document (number 1 on June 4, 2022) to get this approval.

Results

Histopathology of 47 patients was tested, which showed 12 (25.5%) benign serous cystadenomas, 14 (29.8%) serous borderline tumors, and 21 (44.7) invasive serous adenocarcinoma. The mean age was highly significant ($P < 0.001$) and higher in invasive serous adenocarcinoma than benign serous cystadenoma and serous borderline tumor (54.33, 32.166, and 39.57 correspondingly) as in Table 1.

The size of tumors was divided into three categories that showed insignificant association ($P = 0.645$), serous borderline tumors were more presenting in tumor size 1–5 and ≥10 cm (57.1% and 40%, respectively), whereas invasive serous adenocarcinoma were more presenting in 6–9 cm tumor size (52%) as in Table 2.

Immunohistochemical investigation exhibited DKK3 staining was mostly in the cytoplasm of ovarian cells [Figure 1], DKK3 immunoreactive score was significantly high in different types of ovarian tumors, and with more

Table 1: Significant association between ovarian serous tumor and age factor

Parameters		Benign serous cystadenoma no. (%)	Serous borderline tumor no. (%)	Invasive serous adenocarcinoma no. (%)	*P*-value
Age (years)	mean ± SD	32.166 ± 11.91	39.57 ± 12.74	54.33 ± 10	<0.001**
	Median	31.5	40	53	
Age groups (years)	<20	3 (25%)	2 (14.3%)	0	0.013*
	21–30	2 (16.7%)	1 (7.1%)	0	
	31–40	5 (41.7%)	4 (28.6%)	1 (4.8%)	
	41–50	1 (8.3%)	5 (35.7%)	7 (33.3%)	
	51–60	1 (8.3%)	2 (14.3%)	8 (38.1%)	
	61–70	0	0	4 (19%)	
	>70	0	0	1 (4.8%)	

*Chi-square test.

**One-way ANOVA test

Table 2: Association between ovarian serous tumor and neoplasm size

Parameters	Size range of tumor			*P* value
	1–5 cm	6–9 cm	≥10 cm	
Benign serous cystadenoma, no. (%)	4 (26.7%)	8 (32%)	0	0.645*
Serous borderline tumor, no. (%)	4 (57.1%)	4(16%)	6 (40%)	
Invasive serous adenocarcinoma, no. (%)	3 (42.9%)	13(52%)	5 (33.3%)	
Total, no. (%)	7 (100%)	25 (100%)	15 (100%)	

*Kruskal–Wallis test

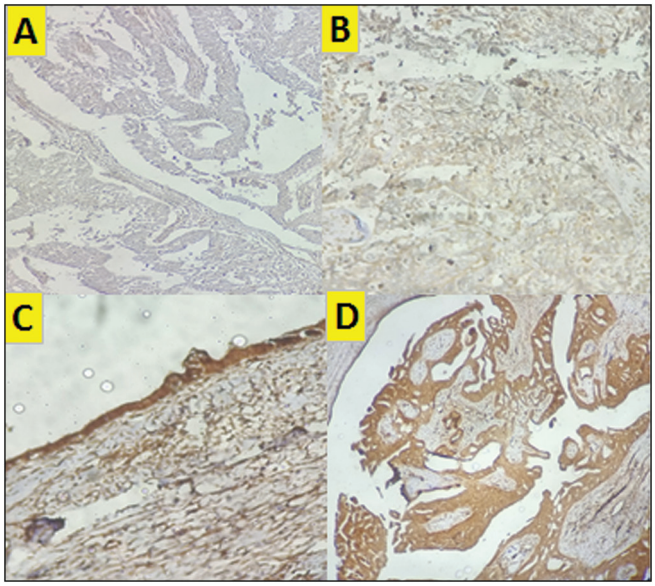

Figure 1: DKK3 expression in the serous epithelial ovarian tumor. (A) Invasive serous adenocarcinoma shows negative staining of DKK3. (B) Invasive serous adenocarcinoma shows +1 staining of DKK3. (C) Benign serous cystadenoma shows +3 staining of DKK3. (d) The serous borderline tumor shows +3 staining of DKK3

percentage of DKK3 Immunohistochemical (+3 and +2) staining in serous cystadenoma. Nearly, 42.9% (9 of 21) of invasive serous adenocarcinomas displayed complete loss of DKK3, whereas 33.3% (7 of 21) showed a +3 DKK3 staining score. Benign serous cystadenomas expressed 50% DKK3 +3 staining, in contrast to serous borderline tumors that were presented with 57.1% of +1 DKK3 staining, as illustrated in Table 3.

Tumor ovarian locations were highly significant, in which unilateral tumor ovarian locations were mostly presented in serous borderline tumors 92.9% (13 of 14), whereas bilateral locations of ovarian tumors were more in invasive serous adenocarcinoma 71.4% (15 of 21) as in Table 4.

Comparing the correlation between immunoreactive DKK3 score level with lateral ovarian location and ovarian neoplasm size [Table 5], there is a positive significant correlation between immunoreactive DKK3 score and ovarian neoplasm size in benign serous cystadenoma, and also there is a positive significant correlation between immunoreactive DKK3 score and ovarian neoplasm location in both serous borderline tumor and invasive serous adenocarcinoma.

Discussion

Ovarian cancer is the fifth most important reason for causing death in females the EOC shows about 90% from the whole ovarian cancer.[30] The majority of EOCs are established with more progressive steps of cancer metastasis and there are unsuccessful chemotherapy drugs for such progressive cancer stages due to the presence of resistance to chemotherapy drugs.[30]

In the current study, there are advanced age groups in invasive serous adenocarcinoma (mean age = 54.33 years old) in contrast to benign serous cystadenoma and serous

Table 3: Immunoreactive score in ovarian serous tumor

Parameters	Immunoreactive score					*P* value
	No staining	+1	+2	+3	Total no.	
Benign serous cystadenoma, no. (%)	0	2 (16.7%)	4 (33.3%)	6 (50%)	12 (100%)	0.045*
Serous borderline tumor, no. (%)	1 (7.1%)	8 (57.1%)	3 (21.4%)	2 (14.3%)	14 (100%)	
Invasive serous adenocarcinoma, no. (%)	9 (42.9%)	3 (14.3%)	2 (9.5%)	7 (33.3%)	21 (100%)	

*Kruskal–Wallis test

Table 4: Distribution of ovarian serous tumor according to lateral location

Parameters	Unilateral location	Bilateral location	*P* value
Benign serous cystadenoma, no. (%)	10 (83.3%)	2 (16.7%)	<0.001*
Serous borderline tumor, no. (%)	13 (92.9%)	1 (7.1%)	
Invasive serous adenocarcinoma, no. (%)	6 (28.6%)	15 (71.4%)	
Total, no. (%)	29 (100%)	18 (100%)	

*Chi-square test

Table 5: The correlation between immunoreactive DKK3 score level with lateral ovarian location and ovarian neoplasm size in all patients with different ovarian tumor types

Parameters		Immunoreactive score	
		R	*P* value
All ovarian tumor types	Lateral ovarian location	0.335	0.154*
	Ovarian neoplasm size	0.75	0.088**
Benign serous cystadenoma	Lateral ovarian location	0.447	0.301*
	Ovarian neoplasm size	1	<0.001**
Serous borderline tumor	Lateral ovarian location	1	0.003*
	Ovarian neoplasm size	0.902	0.113**
Invasive serous adenocarcinoma	Lateral ovarian location	0.73	0.011*
	Ovarian neoplasm size	0.82	0.128**

*Cramer's *V* test

**Gamma test

borderline tumor (mean age = 32.166 and 39.57 years old, respectively), in comparison with Nguyen *et al.* study[28] who showed that 53.2 years old as the mean age group in serous adenocarcinoma, also Kurman[31] study showed that patients with more advanced age associated with high grade serous ovarian cancer.

In this study, DKK3 immunoreactive scores showed a lower significant association in invasive serous adenocarcinoma compared with benign serous cystadenoma and serous borderline tumor. DKK3 immunoreactive +3 score showed a high significant association in benign serous cystadenoma in comparison with serous borderline tumor and invasive serous adenocarcinoma. Nearly, invasive serous adenocarcinoma was 42.9% (9 of 21 patients) absent DKK3 staining and serous borderline tumor showed 7.1% (1 of 14 patients). This result was compatible with Nguyen *et al.*'s[28] study reported that DKK3 +2 immunoreactive score showed a lower highly significant association in invasive adenocarcinoma in comparison with normal, benign adenoma, and borderline tumor also, DKK3 +3 immunoreactive score showed a lower significant association in borderline tumor than that in benign adenoma and invasive adenocarcinoma.

Moreover, the study shows that the DKK3 immunohistochemical staining of different ovarian tumor types was 78.7% (37 of 47 patients). Nguyen *et al.*'s[28] study showed 61.9% of the DKK3 staining score. DKK3 protein is down-regulated at about 40.5%–76.6% in many tumors such as intestine, ovary, and gastric tissue.[25,27,32-38] There is a significant association between ovarian tumors with the lateral location of ovarian tumors, in which the most invasive serous adenocarcinoma presented 71% in both sides of ovaries (bilateral location) and benign serous cystadenoma is found in 83.3% in one side location (unilateral location).

Despite the restrictions associated with this retrospective research and the low number of samples, the study shows a significant association between loss of DKK3 protein expression and different age groups as well as the lateral locations of various ovarian tumors.

Additionally, there is a positive correlation of DKK3 immunohistochemistry with the size of benign serous cystadenomas, as well as the site location of serous borderline tumors and Invasive serous adenocarcinoma.

Conclusion

Serous ovarian tumors have a significant decrease in DKK3 protein expression, especially serous borderline tumors and invasive serous adenocarcinoma. The DKK3 protein expression is affected by age groups, site location of tumor, and not affected by tumor size.

Data availability

Entirely the information necessary to support the findings of this presented research is contained within the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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