Diagnostic Evaluation of Immunohistochemical Expression of p16 and ki67 in Ovarian Serous Neoplasms

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ABSTRACT:

BACKGROUND:

Ovarian serous neoplasms are the most common type of epithelial ovarian tumors, classified as Low-Grade Serous Neoplasia (inclusive of benign serous cyst adenoma, borderline serous tumors, low grade serous carcinoma) and High-Grade Serous Carcinoma. P16 is a cyclin-dependent kinase-IV inhibitor that mediates its action through inhibitory effect on the cell cycle, Ki-67 determine the proliferating cells of the tumor, is strictly connected with cellular cycle.

OBJECTIVE:

The aim of the present study is to diagnostic evaluate of p16 and ki67 protein expression in of ovarian serous tumors in Iraqi patients.

MATERIAL AND METHODS:

52 cases of ovarian serous tumors specimens were studied. Using p16 (Mouse monoclonal p16 antibody, Cytoplasm and Nuclear expression) and ki67 (monoclonal mouse antihuman Ki-67/MIB-1 antibody, Nuclear expression).

RESULTS:

There is a significant correlation of p16 and ki67 expression in ovarian serous tumors. P16 Immune reactive score cases with score 3 of high grade serous carcinoma 69.2%, low grade serous carcinoma 7.7%, borderline serous 15.4%, and no any case of ovarian serous cyst adenoma. Ki67 labeling index expression in all cases of ovarian serous cyst adenoma, 76.92% borderline tumors, 92.96% low grade serous tumors with low labeling index. While 100% high grade serous carcinoma, 23.07% borderline tumors, 7.7% low grade serous carcinoma with high ki67 labeling index.

CONCLUSION:

Significant correlation of p16 expression in ovarian serous tumors, with strong diffuse expression in high grade serous carcinoma. P16 can be used as a diagnostic marker. Ki67 has been reported to be of a diagnostic value in differentiation of low grade serous carcinoma from high grade serous carcinoma.

KEY-WORDS: Ovarian serous neoplasms, P16 and Ki67.

INTRODUCTION:

Ovarian serous neoplasms are the most common type of epithelial ovarian tumors, which derive from mullerian epithelium, make up about one-fourth of all ovarian tumors[1].

Approximately 50% of serous tumors are benign, 15% are borderline, and 35% carcinoma (5-10% low grade serous carcinoma &more than 90% high grade serous).[2] Benign serous cystadenoma are distinguished from serous borderline tumor by the absence of cellular stratification and atypia, borderline serous tumors are distinguished from low-grade serous carcinoma by the absence of stromal invasion.[1,3] LGSC distinguished from the much more common HGSC by their frequent association with serous borderline tumor and a lesser degree of nuclear pleomorphism, lower mitotic rate than HGSC (12 ≤ / 10 HPF) .[1,2,4]

In one study done to describe the epidemiology of the diagnosed cases of ovarian cancer from 2014 to 2017 in Erbil/ Iraq, the result showed that ovarian cancer occurs in old age groups with a percentage of 64%, the most common type is epithelial ovarian cancer with subtype papillary serous adenocarcinoma in 56% .[5]

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$P16^{INK4a}$ is a tumor suppressor protein that is a cyclin-dependent kinase inhibitor, encoded by CDKN2A, within the INK4/ARF tumor suppressor locus on Chromosome 9 (9p21.3)[6], is essential in regulating the cell cycle, and it inactivates cyclin-dependent kinases that phosphorylate retinoblastoma (Rb) gene; therefore p16 can decelerate the cell cycle.[7] P16 overexpression is a surrogate biomarker of HPV infection, which makes it useful in evaluating HPV-associated squamous and glandular neoplasia of the lower gynecologic tract.[7,8] HPV-independent mechanisms of p16 overexpression also exist; so, p16 expression may be observed in tumors that do not necessarily harbor HPV infection, such as high-grade serous carcinoma.[8]

Ki 67 (MIB1) is an excellent immunohistochemical marker to determine proliferating cells of the tumor.[9] The gene for Ki67 protein is located on chromosome 10q25, Ki67 protein expression is strictly connected with cellular cycle.[9,10] It is expressed in all active phases of the cell cycle except in resting cells, the monoclonal Ki-67/MIB-1 antibody reacts with the nuclear Ki-67 antigen expressed in proliferating cells.[10]

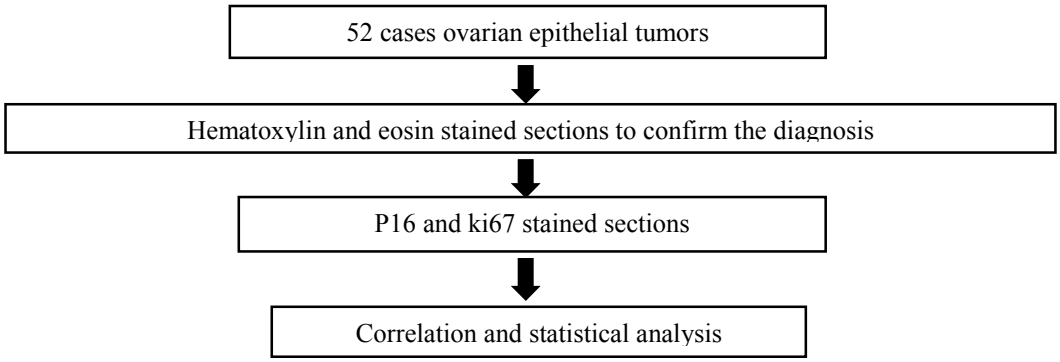
MATERIALS AND METHODS:

1. Sampling of cases: This retrospective cross-sectional study was carried out in Babylon training center for Pathology, during the period from December 2020 through December 2021. Biopsy type for ovarian serous neoplasms cases were excisional biopsy. Reevaluation of all the slides was done by expert pathologist to confirm the histopathological diagnosis. These cases were collected from laboratories of histopathology in Al-Hilla Teaching Hospital, Imam Sadiq Hospital in Babylon, Medical City Educational Laboratories, Private Nursing Home in Baghdad and from some private laboratories in Babylon.

Patients information:

Patients clinicopathological information include tumor types, patient age, tumor size, tumor site, total numbers of specimen. A total of 52 ovarian serous tumor specimens included: (13) Benign ovarian serous cyst adenomas, (13) Borderline ovarian serous tumors, (13) Low grade ovarian serous carcinoma, (13) High grade ovarian serous carcinoma. The specimens were formalin-fixed, paraffin embedded tissue blocks. From each tissue block, 3sections of 5 micrometer thickness were obtained.

Study design diagram



2. Immunohistochemical staining method:

Primary antibody (p16): Mouse monoclonal anti-p16, Cytoplasm and Nuclear expression, 6 ml, PathnSitu ready to use antibodies, Clone:G175-405.

Positive Control: Cervical Carcinoma (Human cervical squamous cell carcinoma). **Negative Controls**: sections were treated similarly with the exception of the primary antibody.

Ki 67 (MIB1): Ki67 antigen immunostaining was carried by standard IHC method and peroxidase-antiperoxidase method using monoclonal mouse antihuman Ki-67/MIB-1 antibody kit, 6 ml, PathnSitu ready to use antibodies (Clone: GM001), (USA). Breast carcinoma was taken as a **positive control** whereas Liver tissue (hepatocytes) was used as **negative control.**

RESULTS:

Table 1: Distribution of ovarian serous tumors in different age groups.

Age by years	Benign serous Cystadenom	Borderline Serous	LGOSC	HGOSC	P value
<20y	2 (15.4)%	1 (7.7)%	-	-	
20_29y	3 (23.07)%	1 (7.7)**%**	-	-	
30_39y	4 (30.76)%	3 (23.07)%	1 (7.7)%	-	
40_49y	3 (23.07)%	6(46.15)**%**	7(53.84)**%**	2 (15.4)**%**	Sig <0.05
50_59y	1 (7.7)%	1 (7.7)%	4(30.76)%	5(38.46)%	
60_69y	-	1 (7.7)**%**	1 (7.7)**%**	5(38.46)%	
>70y	-	-	-	1 (7.7)%	

Age range from (17 – 74) years, the most common age of the patients affected by ovarian serous tumors include of serous cyst adenoma at age range between 30– 39 y (30.76)%, borderline serous tumors between 40_49y (46.15)%, while LGOSC age range 40_49y (53.84%), and HGOSC age 50_69y (76.92%). while only 1 (7.7)% cases was above 70y in HGOSC. P value 0.000(<0.05) is considered a significant correlation.

Table 2: Immunohistochemistry results with regard to p16 IRS (immune reactive score).

IRS score	Serous cyst adenoma	Borderline serous	LGSC	HGSC	P value
0 :NO(0)	10(76.92)%	2 (15.4)%	1 (7.7)%	-	
1: LOW(1-5)	3 (23.07)%	6(46.15)**%**	10(76.92)%	-	
2 :MOD(6-10)	-	3(23.07)%	1 (7.7)%	4(30.8)%	Sig <0.05
3:STRONG(>10)	-	2 (15.4)%	1 (7.7)%	9(69.2)%	

IRS was calculated by multiplying the staining intensity by the percentage of positive cells, resulting in a minimum of IRS 0 and a maximum of IRS 15. HGSC have 9(69.2)% case of score 3, LGSC have only 1 (7.7)% case of strong IRS score3, while borderline serous 2 (15.4)% case of score 3 and no any case of ovarian serous cystadenoma with score 3. The corresponding P-values 0.000(<0.05) is considered statistically significant.

Table 3: Correlation between p16 immunoreactivity score of LGSC & stage.

score	Score0	Score1	Score2	Score 3	Total	P value Not sig 0.171
T1	1 (7.7)%	1 (7.7)%	-	-	2	
T2	-	5(38.46)**%**	-	-	5	
T3	-	4(30.8)%	1 (7.7)%	1 (7.7)%	6	
Total	1	10	1	1	13	

In our study in correlation of p16 IRS and stage of low grade serous carcinoma, we found 1 (7.7)% case stage 3 score 3, 1 (7.7)% case stage 2 score 2 and there is 1 (7.7)% stage1, 5 (38.46)**%** case stage2 & 4 (30.8)% case stage 3 were score 1, the p value between stage and IRS p16 of LGSC 0.171 (>0.05) statistically was not significant.

Table 4:Correlation between p16 immunoreactivity score of HGSC & the stage.

Score	Score0	Score1	Score2	Score3	Total	P vaue
T1	-	-	-	-	-	
T2	-	-	2(15.4)%	-	2(15.4)%	Sig 0.019
T3	-	-	2(15.4)%	9(69.2)%	11(84.61)%	
Total	-	-	4(30.8)%	9(69.2)%	13(100)%	

In our study in correlation of p16 IRS and stage of high grade serous carcinoma, we found 9/13(69.2)%case T3score 3, 2/13(15.4)%case T2& 2/13(15.4)%case T3were score 2. The p value between stage and IRS p16 of HGSC 0.019 (<0.05) was statistically significant.

Table 5: Ki67 labeling index expression in ovarian serous tumors.

Ki67	Ovarian serous cyst adenoma	Borderline serous cyst adenoma	LGSC	HGSC	Total	P value (<0.05) Sig
Low ki67 (LI)	13(100)%	10(76.92)%	12(92.96)%	-	36	
High ki67 (HI)	-	3 (23.07)%	1 (7.7)%	13(100)%	15	

ki67 labeling index (LI) expression in ovarian serous tumors include 13/13 (100)% case with low (LI) in ovarian serous cyst adenoma.10/13 (76.92)% case with low (LI), 3/13(23.07)% case with high (LI) in ovarian serous borderline tumors.12/13(92.96)% case with low (LI) while 1(7.7)% case with high (LI) in ovarian LGSC. No any case of HGSC associated with low (LI) while 13/13(100)% case associated with high (LI). There is significant correlation between ovarian serous tumors and ki67 (LI) P value 0.000 (<0.05).

Table 6: Ki67 labeling index correlation with p16 (IRS) expression in LGSC.

score	Score0	Score1	Score2	Score 3	Total	P value 0.001 Sig
KI67 (LI)	1(7.7)%	10(76.92)%	1 (7.7)%	-	12(92.96%	
KI67 (HI)	-	-	-	1 (7.7)%	1(7.7)%	
Total	1(7.7)%	10(76.92%	1(7.7)%	1 (7.7)%	13(100)%	

In our study of Ki67 labeling index correlation with p16 (IRS) expression in LGSC, there is 1 score0, 10 score1, 1 score1 cases with low KI labeling index, while only 1 case score3 with high KI67 labeling index. P value 0.001(<0.05) consider statistically significant.

Table 7: Ki67 labeling index correlation with p16 (IRS) expression in HGSC.

score	Score0	Score1	Score2	Score 3	Total	P value Not Sig 0.402
KI67(LI)	-	-	-	-	-	
KI67(HI)	-	-	4(30.8)%	9(69.2)%	13(100)%	
Total	-	-	4(30.8)%	9(69.2)%	13(100)%	

In correlation of KI67 labeling index with p16 (IRS) expression in HGSC, there is 4(30.8)% score2,9(69.2)%score 3 with high ki67 labeling index, there is no any case with low ki67 in high grade serous carcinoma. P value 0.402(>0.05) was considered not significant.

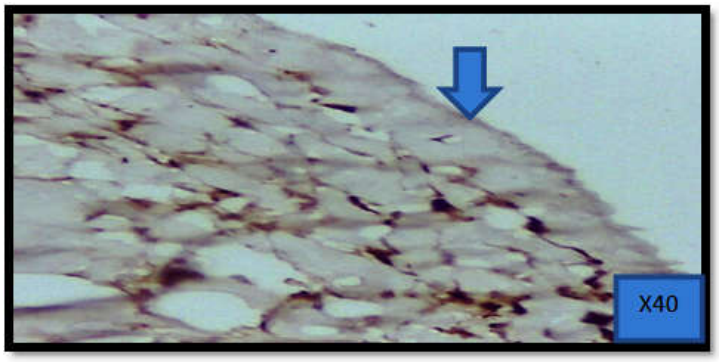


Figure 1: Photomicrograph show ovarian serous cyst-adenoma with negative p16 expression (arrow).

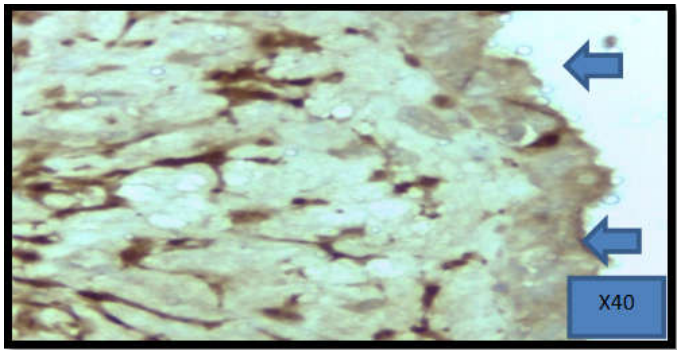


Figure 2: Photomicrograph show ovarian serous cyst-adenoma with low ki67 labeling index expression (arrow).

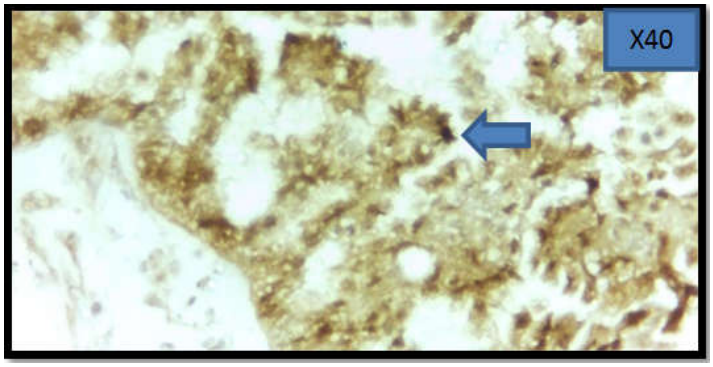


Figure 3:Photomicrograph show borderline serous ovarian tumor with moderate intensity score 2 of p16 expression (arrow).

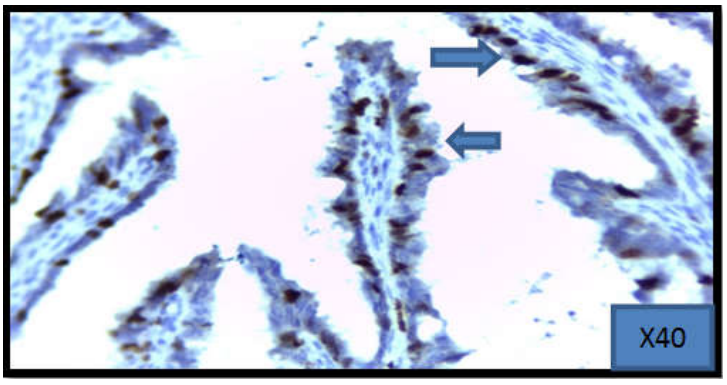


Figure 4: Photomicrograph show borderline serous ovarian tumor with high ki67 labeling index (arrow).

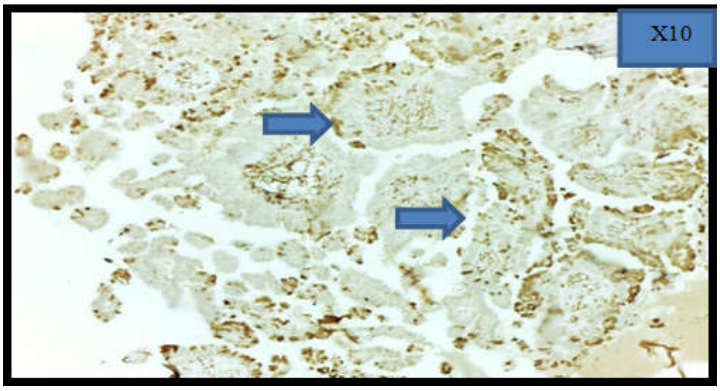


Figure 5: Photomicrograph show low grade serous ovarian cancer with weak intensity score 1 and focal p16 expression (arrows).

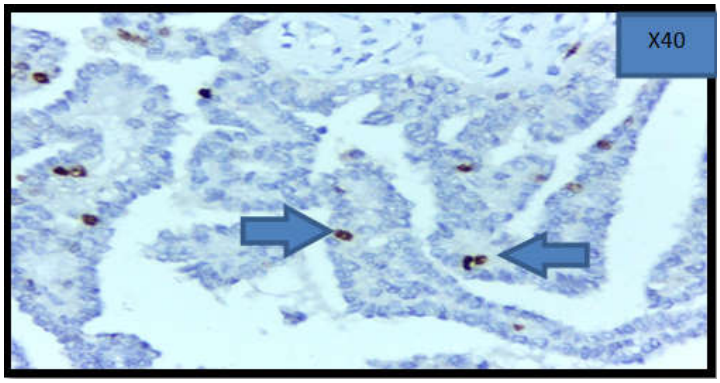


Figure 6 :Photomicrograph shows low grade ovarian serous carcinoma with low mitotic activity low ki 67 labeling index (arrows).A(X10),B(X40).

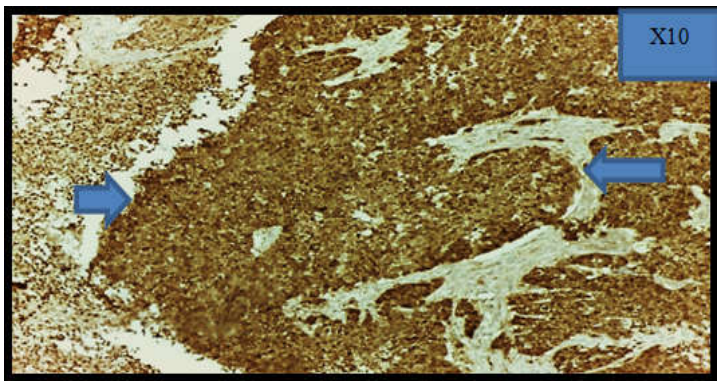


Figure 7: Photomicrograph shows high grade ovarian serous carcinoma p 16 with diffuse positivity and strong intensity score 3+(arrow).

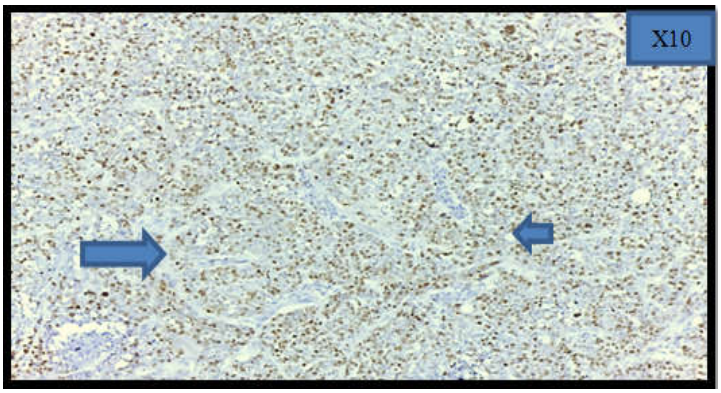


Figure 8: Photomicrograph shows high grade ovarian serous carcinoma with high mitotic activity high ki67 labeling index(arrow). A(X10),B(X40).

DISCUSSION:

It had been found in the current studythat there was a significant correlation between age groups & serous ovarian tumors histological types p value 0.000(<0.05), this finding agrees with a study done by Purti Agrawal[11] predominant of benign ovarian serous cyst occurs (30-40)y, while malignant serous (40-50 to more than60) y, also agrees with a study done by Shahhnaz Begum[12],and agrees with a study done by Fei Deng[13] p value 0.02 signification correlation ovarian LGSC&HGSC with age,while disagrees with a study done by Mohamed Y Ali [14] in which p value 0.09 is not significant . These differences may be related to different population, race and also due to different in number of cases. It had been found in the current study that there is a significant correlation of p16 expression in ovarian serous tumors. This significant correlation of p16 expression in ovarian serous tumors according to distribution, intensity and immune reactive score in our study agrees with first similar study done by C J O'Neill [15] in 2007 there is significant correlation for distribution, intensity and composite score between the borderline tumors and HOSC, also between the LOSC and HOSC group. it agrees with a study done H.O. Nazlioglu[16] in 2010; the difference between the borderline tumors compared to the benign group (p <0.001), borderline tumors and ovarian serous carcinomas was statistically significant (p < 0.001). It also agrees to the following study done by Peter W. Schlosshauer[17] in 2011, HGSC when compared with both typical SBTs and LGSC statistically significant correlation; agrees with a study done by Luis Felipe Sallum[18] in 2018 p value (< 0.001) significant correlation of HGSC compared with LGSC, and agrees with a study by Surg Capt V. Manu[19] in 2018 that there is a significant correlation of p16 with ovarian serous tumors regarding to distribution, intensity and IRS. The correlation of p16 expression with stage of LGSC; we found p value 0.171 statistically was not significant, this agrees with a study done by Rambau PF et al[20] 2018 p value was 0.52 statistically not significant. We found in our study in correlation of p16 and stage of HGSC p 0.019 statistically significant, this result disagrees with a similar study done by Rambau PF et al[20] 2018 p was 0.65 statistically not significant. There is very limited study to compare with we didn't find any other similar study. In correlation of ki 67 LI with ovarian serous tumors, we found significant correlation p value 0.000, this agrees with a study done by Monisha Choudhury [21] 2011 also significant correlation was observed in a study done by Dinka Sundov[9] 2013 p value < 0.001in correlation of ki67(LI) with low and high grade ovarian serous carcinoma, also agrees with a study done by Mohamed Y Ali[14] 2019, significant correlation of ki67 in benign serous tumors with ovarian serous carcinoma. Also, significant correlation of ki67 in LGSC with HGSC in a study done by Sheronica Laishram[10] 2019 also agrees with a study done by Luis Felipe Sallum[22], p value 0.01consider significant correlation. It had been found in our study that there is a significant correlation of KI67 labeling index with p16 in LGSC P value 0.001(<0.05) and there is no significant correlation of Ki67 labeling index with p16 in HGSC P value 0.402(>0.05), this agrees with a study done by Pawel Surowiak[23], there is no significant correlation between of KI67 labeling

index with p16 in HGSC but disagrees with LGSC of KI67 labeling index with p16 with no significant correlation, this may be related to technical error, different in numbers of specimens and in no any other study found .

CONCLUSION:

There is a significant correlation of p16 expression in ovarian serous tumors, with strong diffuse expression in high grade serous carcinoma compared with low grade serous carcinoma, borderline serous and benign serous tumors, this is in keeping with a different underlying pathogenesis for these neoplasms. P16 can be used as a diagnostic marker. Ki67 determines proliferative activity of the tumors, has been reported to be of a diagnostic value in differentiation of low grade serous carcinoma from high grade serous carcinoma.

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