ORIGINAL PAPER

CLINICO-MORPHOLOGICAL ASPECTS AND NEW IMMUNOHISTOCHEMISTRY CHARACTERISTICS OF OVARIAN HIGH-GRADE SEROUS CARCINOMA

Manuela POPA[1], Adelina BIRCEANU COROBEA[2]✉, Monica M. CIRSTOIU[3], Octavian MUNTEANU[3], Adriana E. NICA[4], Mariana COSTACHE[1,5], Maria SAJIN[1,5]

[1] Department of Pathology, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] Department of Pathology, „Sfanta Maria" Clinical Hospital, Bucharest, Romania
[3] Department of Obstetrics and Gynecology, University Emergency Hospital Bucharest, Romania
[4] Department of Anesthesiology and Intensive Therapy, University Emergency Hospital Bucharest, Romania
[5] Department of Pathology, University Emergency Hospital Bucharest, Romania

ABSTRACT

Introduction High-grade serous carcinoma of the ovary is an aggressive form of cancer, with unknown precursor lesions and often delayed diagnosis because of non-specific, mild symptoms.

Objective We performed a clinical-pathological study of ovarian high-grade serous carcinomas, in order to evaluate morphological and new immunohistochemistry characteristics of this malignancy.

Methods This is a retrospective study of 10 cases of ovarian high-grade serous carcinoma. We evaluated patients' age, symptoms at presentation, macroscopic aspects, bilateral involvement, microscopic features: papillary/solid areas, mitotic index, psammoma bodies, tumoral extension, lymph node metastasis, immunohistochemistry markers: CD44, ER, AR, Ki67 index.

Results Mean age was 56.9 years old. Tumors were bilateral in 50% of cases. Only 30% were limited to the ovary. Maximum tumor diameter was 16 cm. Solid component in a proportion of 50-95% was more

RÉSUMÉ

Aspects clinico-morphologiques et caractéristiques nouvelles immunohistochimiques du carcinome séreux ovarien à haut degré

Introduction Le carcinome séreux à haut degré de l'ovaire est une forme agressive de cancer avec des lésions précurseurs inconnues et un diagnostic souvent différent en raison de symptômes mineurs non spécifiques.

Objectifs Nous avons effectué une étude clinico-pathologique sur les carcinomes séreux à haut degré ovariens afin d'évaluer les points saillants morphologiques et immunohistochimiques de cette malignité.

Méthodes Ceci est une étude rétrospective de 10 cas de carcinome séreux ovarien de haut grade. Nous avons évalué l'âge, les symptômes à la présentation des patients, les aspects macroscopiques, l'atteinte bilatérale, les caractéristiques microscopiques: zones papillaires/solides, index mitotique, corps du psammome,

✉ Address for correspondence: Adelina BIRCEANU COROBEA
Department of Pathology, „Sfanta Maria" Clinical Hospital, Bucharest, Romania
Address: Ion Mihalache Blvd, 37-39, Bucharest, 011172, Romania
adelinabirceanu@gmail.com; phone 0040765010097

characteristic. Most tumors had a mitotic index of 30-50 mitosis/10HPF (70% of cases). 20% of cases contained psammoma bodies. 2 cases out of 7 had lymph node metastasis. We noticed one case with pleural metastasis (M1). We observed AR<10% was characteristic of 90% of tumors. Ki67 index >80% was noticed in 30% of cases. CD44 was positive in 50% of cases and one case had diffuse positivity of CD44 in corpus luteum cells near the tumoral bed.

Conclusions The majority of patients with ovarian high-grade serous carcinomas presented with extraovarian extension and were characterized by high mitotic index, rare presence of psammoma bodies, AR expression <10%, novel marker CD44 positive in 50% of cases and curious positivity in corpus luteum cells associated with the tumor.

Keywords: high-grade serous carcinoma, CD44, corpus luteum cells.

Abbreviation list
AR = androgen receptor
BRCA1/2 = breast cancer protein 1/2 encoded by tumor suppressor gene BRCA1, BRCA2 respectively
CA125 = cancer antigen 125
CD44 = cluster of differentiation 44
CK7= cytokeratin 7
EMA = epithelial membrane antigen
EphB1 = ephrin-B1 receptor
ER = estrogen receptor
GRIM-19 = homologous protein to NADH dehydrogenase 1 alpha subcomplex subunit 13
HE = hematoxylin-eosin stain
IHC = immunohistochemistry stain
HG = high-grade
HPF = high power field
IKK2 =inhibitor of nuclear factor kappa-B kinase subunit beta
Ki67 = Anti-human Ki-67 Antibody = proliferation index
NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells
p53 = tumor protein p53, encoded by tumor suppression gene TP53
PTPN13 = Tyrosine-protein phosphatase non-receptor type 13
WHO = World Health Organization
WT1 = Wilms tumor protein, encoded by WT1 gene

extension tumorale, métastases ganglionnaires, marqueurs immunohistochimiques: CD44, ER, AR, indice Ki67.

Résultats L'âge moyen était de 56,9 ans. Les tumeurs étaient bilatérales dans 50% des cas. Seulement 30% étaient limités à l'ovaire. Le diamètre maximal de la tumeur était de 16 cm. La composante solide en proportion de 50 à 95% était plus caractéristique. La plupart des tumeurs avait un index mitotique de 30-50 mitoses / 10HPF (70% des cas). 20% des cas contenaient des corps de psammoma. 2 cas sur 7 avaient une métastase ganglionnaire. Nous avons remarqué un cas de métastase pleurale (M1). Nous avons observé que l'AR <10% était caractéristique de 90% des tumeurs. Indice Ki67> 80% a été noté dans 30% des cas. Le CD44 était positif dans 50% des cas et un cas présentait une positivité diffuse du CD44 dans les cellules du corps jaune près du lit tumoral.

Conclusions Nous avons trouvé un âge moyen inférieur. La majorité des patients présentaient une extension extra-ovarienne et se caractérisaient par un index mitotique élevé, une présence rare de corps psammomateux, une expression AR <10%, un nouveau marqueur CD44 positif dans 50% des cas avec une positivité singulière dans les cellules du corps jaune associées à une tumeur.

Mots-clés: carcinome séreux à haut degré, CD44, cellules du corps jaune.

INTRODUCTION

High-grade (HG) serous carcinoma is an aggressive malignant epithelial tumor of the female gonad, although often with a delayed diagnosis because of non-specific, mild symptoms, responsible for about

225,000 new cases worldwide every year and 140,000 deaths[1].

Ovarian serous carcinoma has been for a long time considered a single entity, but studies of their genetic profile, correlated with different morphologic features and clinical outcome, has led to their

classification in a two-tier system, as described by Shih et al in their published work, into low-grade serous carcinoma and high-grade serous carcinoma[2,3].

Low-grade serous carcinoma is characterized by low-grade cytological atypia, with known precursor lesion, that evolves slowly and has a better prognosis on survival, whereas high-grade tumor has moderate to severe atypia, higher mitotic index, rapid evolution and unknown precursor lesions[1,4,5]. Over time, numerous studies have postulated potential preneoplastic conditions, secretory cell outgrowth, p53 signature, serous tubal intraepithelial carcinoma[6,7].

Immunohistochemistry tests and molecular assays have been used for confirmation of the diagnosis and to differentiate between the two. The immunohistochemical markers in current use are CK7, EMA, WT1, p53, CA125, Ki67 and calretinin[4,8]. Also, new markers have been tested, such as GRIM-19, NF-κB, IKK2, PTPN13, EphB1 protein[9-11]. The most frequent altered genes described for high-grade serous carcinoma are TP53 and BRCA1 and 2[12,13].

Better survival prognosis factors for these patients are younger age at diagnosis, earlier clinicopathologic stage, lower grade, the absence of ascites, optimal cytoreduction at primary surgery, the absence of germline BRCA mutations[14,15].

THE OBJECTIVE OF THE STUDY

Our objective was to perform a clinicopathological study of ovarian high-grade serous carcinomas in order to evaluate detailed clinico-morphological aspects and new immunohistochemistry characteristics of this aggressive disease of the female reproductive system.

MATERIAL AND METHODS

We performed a retrospective study of 10 cases of ovarian high-grade serous carcinoma diagnosed at the University Emergency Hospital Bucharest, Romania. The data were obtained from the Pathology Department records and from the hospital's database. We analyzed the hematoxylin-eosin permanent stains and performed immunohistochemical stains on paraffin-embedded tissue blocks. The HE and IHC stains were analyzed under the optical microscope and photographed using microscope photo acquisition system. The data were introduced in Microsoft Office Excel 2013 and Epi Info 7. The level of confidence interval was 95%, using Fisher's exact test to obtain the *p* value for statistically significant correlation. The parameters evaluated were: patients' age, symptoms at presentation, tumor macroscopic

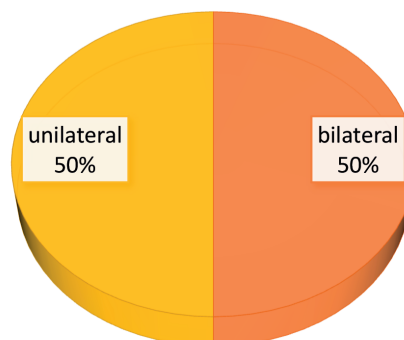## TUMOR LOCALIZATION IN OVARIAN HG SEROUS CARCINOMA

Fig. 1. Tumor localization regarding bilateral involvement

aspects, bilateral ovarian involvement, microscopic features such as: papillary/solid areas ratio, the mitotic index, presence of psammoma bodies, tumoral extension (pT), lymph node metastasis (pN), expression of the following immunohistochemistry markers: CD44, ER, AR, Ki67 index.

RESULTS

The mean age at diagnosis for patients with ovarian HG serous carcinoma was 56.9 years old.

The most frequent clinical symptoms were abdominal pain and abdominal distension (80%). Other symptoms included: anorexia, nausea, vomiting, fever, diarrhea, constipation, vaginal bleeding.

One case was diagnosed incidentally on a routine pelvic ultrasonography, as the patient had no previous complaints.

After clinical examination, ultrasonography and imaging, the patients were submitted to surgery for total hysterectomy with bilateral adnexectomy, and additional lymphadenectomy, or colectomy for tumors extended to the colon.

Tumors were bilateral in 50% of cases (Fig. 1):

Only 3 cases (30%) were limited to the ovary (Fig. 2). The maximum tumor diameter was 16 cm, found in 2 cases, but both were in the pT1a stage.

Solid component in proportion of 50-95% was characteristic of the gross appearance (Fig. 3). Papillary/solid areas ratio ranged from 1:3 to >1:5.

Most HG tumors had a mitotic index of 30-50 mitosis/10HPF (70% of cases).

We observed the presence of psammoma bodies in 20% of cases.

The lymph nodes status evaluation was possible only in 7 cases, from which 2 cases were positive for tumor metastasis – Fig. 4.
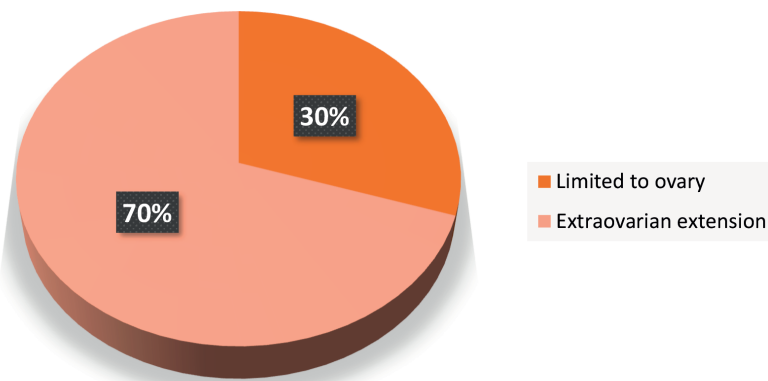
Tumor extension in ovarian HG carcinoma

Limited to ovary
Extraovarian extension

30%
70%

Fig. 2. Tumor extension in ovarian HG carcinoma
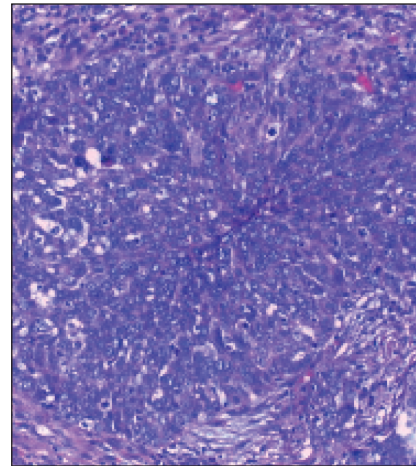
Fig. 3. Microscopic solid area of HG serous carcinoma of the ovary, HE × 20
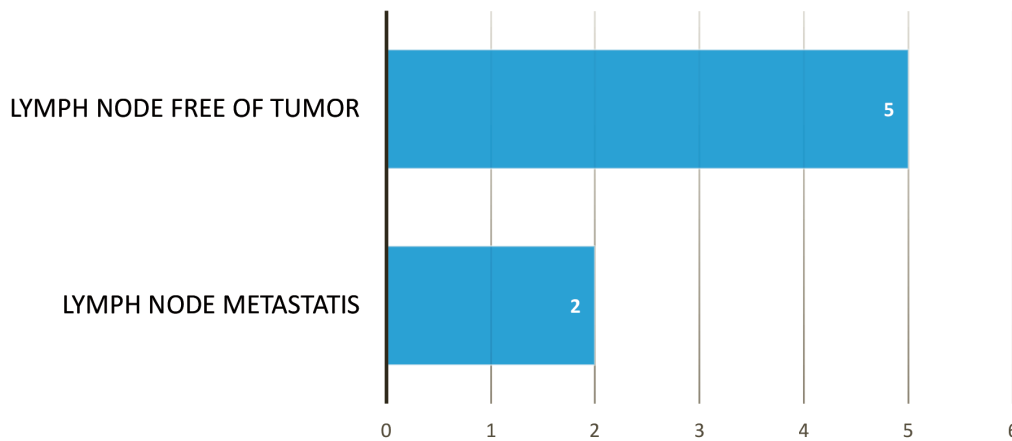
Lymph node status

LYMPH NODE FREE OF TUMOR — 5
LYMPH NODE METASTATIS — 2

Fig. 4. Lymph node status

CD44 EXPRESSION IN HG SEROUS CARCINOMA

CD44 negative — 5
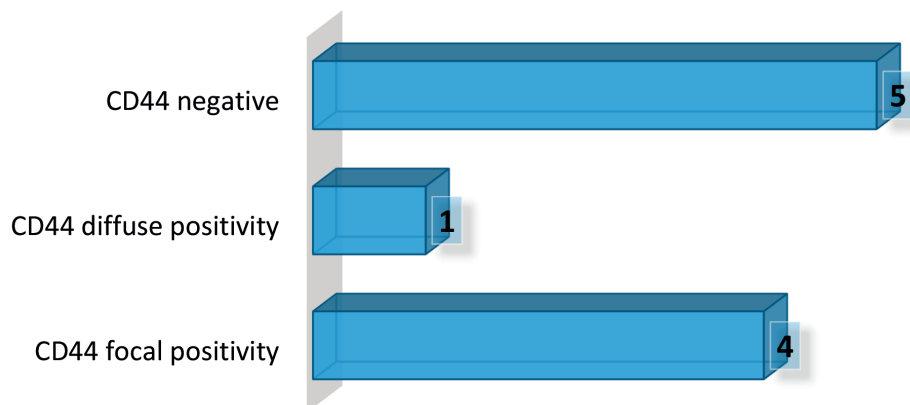CD44 diffuse positivity — 1
CD44 focal positivity — 4

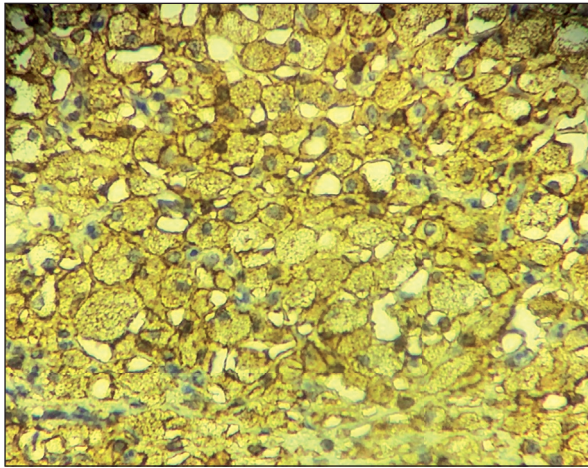**Fig. 5.** CD44 expression in ovarian HG serous carcinoma

Fig. 6. CD44 diffuse positivity in corpus luteum cells of an ovary with HG serous carcinoma, IHC × 40

In the studied group, we noticed one case with pleural metastasis that corresponded to M1 – pTNM.

We observed that AR<10% was characteristic of 90% of tumors. Ki67 index >80% was noticed in 30% of the cases, but it was not associated with AR<10% (p=0.13).

CD44 was positive in 50% of the cases, from which 4 cases with focal positivity and one case with diffuse positivity – Fig. 5.

We also found one case with diffuse positivity of CD44 in corpus luteum cells near the tumoral bed in a high-grade serous carcinoma where tumor cells were negative for CD44 – Fig. 6.

CD44 positivity does not correlate with tumor bilaterality (p=0.5) or with advanced stage disease (p=0.5).

Discussion

The mean age at diagnosis for patients included in our study was 56.9 years old, whereas the mean age reported by the World Health Organization (WHO) is 63 years old[1]. Studies have shown that advanced age ≥ 65 years was correlated with suboptimal surgery outcome[16].

Tumors were bilateral in 50% of cases. This is just below the reported value of two-thirds of the cases for advanced stage cases[4].

Most HG tumors in our cases had a mitotic index of 30-50 mitosis/10HPF. High-grade serous carcinomas have a higher mitosis number compared to low-grade serous carcinoma, that usually has less than 12 mitosis/10HPF[1].

Psammoma bodies are concentric lamellated calcified structures, observed most commonly in papillary thyroid carcinoma, meningioma, and serous carcinoma of the ovary[17]. We observed the presence of psammoma bodies in 20% of cases approximately as described by other studies[4]. The formation of psammoma bodies is associated with increased apoptotic tumor cell death and long-term survival[18].

The prognostic relevance of lymph node metastases in primary ovarian cancer is unclear, although seen as an important prognostic factor for advanced-stage ovarian cancer, there is contradictory data on survival after systematic lymphadenectomy versus bulky node resection[19-22].

We observed that AR<10% is characteristic of 90% of tumors. Previous studies have shown that AR negativity is associated with high-grade tumors, together with the decrease of expression associated with recurrent disease and poor prognosis[23-25]. The androgen receptor also decreases after chemotherapy[26].

We found Ki67 index >80% was noticed in 30% of cases. Higher Ki67 levels are associated with advanced FIGO staging in the literature[27].

CD44 is a glycoprotein receptor that is activated by binding to its major ligand hyaluronic acid and also stated as a stem cell marker[28,29]. Studies in the literature reported positivity for CD44 in the majority of epithelial ovarian carcinomas[29]. CD44 positivity has been associated with advanced stage disease and poor 5-year overall survival[30]. In our study, CD44 was positive in 50% of cases, from which 4 cases with focal positivity and one case with diffuse positivity.

Also, CD44 positivity did not correlate with advanced stage disease (p=0.5), unlike other previous studies[30].

Conclusions

We have found a lower mean age of patients with high-grade tumors. The majority of ovarian high-grade serous carcinoma presented with extraovarian extension at diagnosis and were characterized by high mitotic index, weak presence of psammoma bodies, AR expression <10%, novel marker CD44 positive in 50% of cases and curious positivity in corpus luteum cells associated with the tumor. Further studies are necessary to investigate the presence and role of CD44 and luteal cells in oncogenesis and progression.

Compliance with Ethics Requirements:

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study"

REFERENCES

1. Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO Classification of Tumors of Female Reproductive Organs. 4th Edition, IARC, Lyon, 2014.
2. Shih IeM, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. *Am J Pathol* 2004, 164:1511–1518.
3. Malpica A, Deavers MT, Lu K, et al. Grading ovarian serous carcinoma using a two-tier system. *Am J Surg Pathol* 2004;28(4):496-504.
4. Kurman RJ, Ellenson LH, Ronnett B. Blaustein's Pathology of the Female Genital Tract. Sixth Edition, Springer Science+Business Media, LLC 2011.
5. Sundov D, Caric A, Mrklic I, et al. P53, MAPK, topoisomerase II alpha and Ki67 immunohistochemical expression and KRAS/BRAF mutation in ovarian serous carcinomas. *Diagn Pathol* 2013;8:21.
6. Kobayashi H, Iwai K, Niiro E, et al. The conceptual advances of carcinogenic sequence model in high-grade serous ovarian cancer. *Biomed Rep* 2017;7(3):209-213.
7. Nakamura K, Nakayama K, Ishikawa N, et al. Reconstitution of high-grade serous ovarian carcinoma from primary fallopian tube secretory epithelial cells. *Oncotarget.* 2017;9(16):12609-12619.
8. Köbel M, Rahimi K, Rambau PF, et al. An immunohistochemical algorithm for ovarian carcinoma typing. *Int J Gynecol Pathol* 2016; 35(5): 430–441.
9. Ilelis F, Do Amaral NS, Alves MR, et al. Prognostic value of GRIM-19, NF-κB and IKK2 in patients with high-grade serous ovarian cancer. *Pathol Res Pract* 2018;214(2):187-194.
10. D'Hondt V, Lacroix-Triki M, Jarlier M, et al. High PTPN13 expression in high grade serous ovarian carcinoma is associated with a better patient outcome. *Oncotarget.* 2017;8(56):95662-95673.
11. Wang H, Wen J, Wang H, et al. Loss of expression of EphB1 protein in serous carcinoma of ovary associated with metastasis and poor survival. *Int J Clin Exp Pathol* 2013;7(1):313-21.
12. Lee SH, Kim H, Kim WY, et al. Genetic alteration and immunohistochemical staining patterns of ovarian high-grade serous adenocarcinoma with special emphasis on p53 immunostaining pattern. *Pathol Int* 2013; 63(5):252-9.
13. Dong A, Lu Y, Lu B. Genomic/epigenomic alterations in ovarian carcinoma: translational insight into clinical practice. *J Cancer* 2016; 7(11): 1441–1451.
14. Hoppenot C, Eckert MA, Tienda SM, et al. Who are the long-term survivors of high grade serous ovarian cancer? *Gynecol Oncol* 2018; 148(1):204-212.
15. Petrillo M, Marchetti C, De Leo R, et al. BRCA mutational status, initial disease presentation, and clinical outcome in high-grade serous advanced ovarian cancer: a multicenter study. *Am J Obstet Gynecol.* 2017;217(3):334.e1-334.e9.
16. Deng F, Xu X, Lv M, et al. Age is associated with prognosis in serous ovarian carcinoma. *J Ovarian Res* 2017;10(1):36.
17. Das DK. Psammoma body: a product of dystrophic calcification or of a biologically active process that aims at limiting the growth and spread of tumor? *Diagn Cytopathol* 2009;37(7):534-41.
18. Motohara T, Tashiro H, Miyahara Y, et al. Long-term oncological outcomes of ovarian serous carcinomas with psammoma bodies: a novel insight into the molecular pathogenesis of ovarian epithelial carcinoma. *Cancer Sci* 2010;101(6):1550-6.
19. Bachmann C, Bachmann R, Fend F, et al. Incidence and impact of lymph node metastases in advanced ovarian cancer: implications for surgical treatment. *J Cancer* 2016; 7(15): 2241–2246.
20. Bachmann C, Bachmann S, Fehm T, et al. Nodal status-its impact on prognosis in advanced ovarian cancer. *J Cancer Res Clin Oncol* 2012; 138(2):261-7.
21. Panici PB, Maggioni A, Hacker N. Systematic aortic and pelvic lymphadenectomy versus resection of bulky nodes only in optimally debulked advanced ovarian cancer: a randomized clinical trial. *J Natl Cancer Inst* 2005; 97(8):560-6.
22. Pereira A, Pérez-Medina T, Magrina JF, et al. The role of lymphadenectomy in node-positive epithelial ovarian cancer. *Int J Gynecol Cancer* 2012;22(6):987–92.
23. Elattar A, Warburton KG, Mukhopadhyay A, et al. Androgen receptor expression is a biological marker for androgen sensitivity in high grade serous epithelial ovarian cancer. *Gynecol Oncol* 2012; 124(1):142-7.
24. Jönsson JM, Arildsen NS, Malander S, et al. Sex steroid hormone receptor expression affects ovarian cancer survival. *Transl Oncol* 2015; 8(5): 424–433.
25. Feng Z, Wen H, Ju X, et al. Hormone receptor expression profiles differ between primary and recurrent high-grade serous ovarian cancers. *Oncotarget* 2017;8(20):32848-32855.
26. Zhu H, Zhu X, Zheng L. The role of the androgen receptor in ovarian cancer carcinogenesis and its clinical implications. *Oncotarget* 2017; 8(17): 29395–29405.
27. Mahadevappa A, Krishna SM, Vimala MG. Diagnostic and prognostic significance of Ki-67 immunohistochemical expression in surface epithelial ovarian carcinoma. *J Clin Diagn Res* 2017;11(2):EC08-EC12.
28. Basakran NS. CD44 as a potential diagnostic tumor marker. *Saudi Med J* 2015; 36(3): 273–279.
29. Sacks JD, Barbolina MV. Expression and function of CD44 in epithelial ovarian carcinoma. *Biomolecules* 2015; 5(4): 3051–3066.
30. Lin J, Ding D. The prognostic role of the cancer stem cell marker CD44 in ovarian cancer: a meta-analysis. *Cancer Cell Int* 2017;17:8.