FREQUENCY OF NAPSIN A POSITIVITY IN OVARIAN CLEAR CELL CARCINOMA AND SEROUS CARCINOMA

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ABSTRACT

Objective: To differentiate ovarian clear cell carcinoma and serous carcinoma by expression of Napsin A positivity.

Study Design: Cross-sectional & descriptive study.

Place and Duration of Study: The study was conducted in Shaukat Khannam Memorial cancer hospital from 2016 to 2017.

Material and Methods: A total of n=59 cases of previously diagnosed cases of clear cell carcinoma and high grade serous carcinoma were selected for this study. The slides were reviewed by an expert panel. Napsin A (MRQ-60) Ventana monoclonal antibody was applied to all the cases. Weak to strong membranous staining in more 5% tumor cells was considered positive.

Results: Mean age of patients in the study was 46.25 ± 11.1 years. There were n=33 (55.9%) cases of serous carcinoma, n=23 (38.9%) cases of clear cell carcinoma and n=3 (5.08%) cases had biphasic morphology. CK7 was applied and showed positive expression in all the cases. Napsin A was found positive in all the cases of clear cell carcinoma as compared to only n=1 (3%) high grade serous carcinomas and none of the mixed tumors. The relationship was considered significant with a *p*-value of <0.05.

Conclusion: Expression of Napsin A showed a significant difference in high grade serous carcinoma and clear cell carcinoma. Therefore, Napsin A was found a sensitive and specific marker for the diagnosis of ovarian clear cell carcinoma and can reliably differentiated it from high grade serous carcinoma. Current research recommends a study with large sample size to insight the expression of Napsin A and other immunohistochemical stains.

Keywords: Clear cell carcinoma ovary, High grade serous carcinoma,Napsin A

INTRODUCTION

Ovarian carcinoma is the seventh most common cancer in women worldwide with 239,000 cases diagnosed in 2012. While epithelial ovarian cancers are represent 90% of all ovarian cancers. The most common epithelial tumor is the serous carcinoma, followed by endometrioid carcinoma and clear cell carcinoma. Other important epithelial neoplasia includes Brenner and Mucinous carcinomas[1]. High grade serous carcinomas are the most common malignant epithelial tumor accounting for 86% of the FIGO grade III-IV tumors. These tumors are commonly associated with BRCA ½ mutations and is also associated with oestrogen hormonal therapy. Mean age of diagnosis is 63 years. The tumors present as variable ovarian masses, are frequently bilateral and show a solid-cystic cut surface with areas of papillary growth. Necrosis and hemorrhage are commonly seen. Histologically, serous tumors show papillary, glandular and cribriform patterns. Nuclei are large, hyperchromatic, pleomorphic with prominent nucleoli and psammoma bodies. The tumor cells frequently express WT1 with aberrant expression of P53. These patients are treated with debulking and cytotoxic chemotherapy[2,3]. Clear cell carcinomas have a mean age of 33% of FIGO grade I-II tumors. Patients have a mean age of 55 years, and the tumor is commonly associated endometriosis, paraneoplastic hypercalcemia and venous thromboembolism. The tumors are unilateral, large masses with solid and cystic cut surface with pale yellow nodules. Endometriotic cyst is commonly

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associated with them. The tumor cells are arranged in a tubulocystic, papillary and sold pattern. Tumor cells are polygonal to cuboidal, with clear to eosinophilic cytoplasm and hyper-chromatic nuclei showing hobnailing. The clear cells have glycogen-rich cytoplasm which is demonstrated by PAS/PASD. The tumor is commonly associated with Lynch syndrome (mutations in MSH2). Most common mutations identified include ARID1A, PIK3CA and PTEN[4,5]. Both these ovarian tumors exist in the same demographic population and might have overlapping histologies. Most common IHC markers used to differentiate the two tumors include WT1 and P53 over expression in serous carcinomas, and HNF 1B and NapsinA positivity in ovarian carcinomas. Clear cell carcinomas are resistant to therapy while serous carcinomas respond at least initially[6-8]. NapsinA, is a functional aspartic proteinase that is expressed in the lung parenchyma in type II pneumocytes and proximal convoluted tubules in the kidney. The gene NAPA A has been detected in chromosome 19q and corresponding protein. It promotes resistance to Cis-platin. Napsin A is overexpressed in adenocarcinomas of lung, papillary renal cell carcinoma, thyroid carcinoma and clear cell tumors of gynecological tract[9,10]. Differentiating High grade serous carcinomas and Clear cell carcinomas is extremely important as clear cell carcinomas display resistance to platinum-based therapy. In this study we are comparing the expression of Napsin A among high grade serous carcinomas and clear cell carcinomas of the ovary.

MATERIAL AND METHODS

This cross-sectional and descriptive study designed and conducted in Shaukat Khanum Cancer Hospital and Research Centre. A total of n=59 cases of ovarian carcinoma in between 2016-2017 were included by non-probability convenience sampling. Out of which n=33 were diagnosed cases of serous carcinoma, n=23 are of clear cell carcinoma and n=3 tumors with biphasic morphologies (clear cell and serous carcinoma) were retrieved from the electronic database. Napsin A (MRQ-60) mouse monoclonal antibody was used to stain these sections.The expression of the protein was assessed by a panel of histopathologists with interest in gynecological pathology. A positive result was interpreted as weak to strong membranous staining in >5% of tumor cells. A negative result was considered as absence of staining. Current data were analyzed using SPSS version 20.0. However, mean, median, mode and standard deviation were calculated for age parameter. The significance of difference of expression of Napsin A between high grade serous carcinomas and clear cell carcinomas was calculated using Chi-square test. Whereas, *p*-values ≤0.5 considered as significant.

Table: Clinical and morphological data stratification according to tumor subtype.

Subtype	Mean age (years) (S.D)	Tumor size (cm) (S.D)	Capsule Rupture (%)	Surface deposits (%)	Fallopian tube involvement (%)	PT1 stage of tumor	pT 2	pT3
Clear cell carcinoma	46.25 ± 111	10.85 ± 5.2m	1 (4.5)	6 (54%)	0 (0%)	14 (37%)	04 (33%)	0
High grade serous carcinoma			18 (54%)	18 (72%)	8 (14%)	23 (61%)	07 (58%)	8 (89%)
Tumors with biphasic morphology			2 (66%)	2 (66%)	0 (0)	1 (3%)	1 (8%)	1 (11%)

Note: These values were calculated data. All data was not available in every case.

RESULTS

Mean age of patients was 46.25 ± 11.1 years. Out of total n=59 cases, n=6 (10.1%) of the patients were in the third and fourth decades of life respectively. Majority of the patients, n=47 (79.6%) were 40 years or older. Mean size of the tumor was 10.85 ± 5.2cm. The tumor involved left ovary in n=5 (11.6%) of cases, the right ovary in

n=13 (30.2%) of the cases and both the ovaries in n=25 (58.1%) of the cases. Although, data was not available in n=16 cases. There were n=33 (55.9%) cases of serous carcinoma, n=23 (38.9%) cases of clear cell carcinoma and n=3 (5.08%) cases had biphasic morphology. Furthermore, morphology carcinoma surface deposits were present in n=6 ((54%) cases. Maximum surface deposit is seen in high grade serous carcinoma n=18 (72%) while the minimum was observed in tumors with biphasic morphology n=2 (66%). Surprisingly fallopian tube involvement was found in n=8

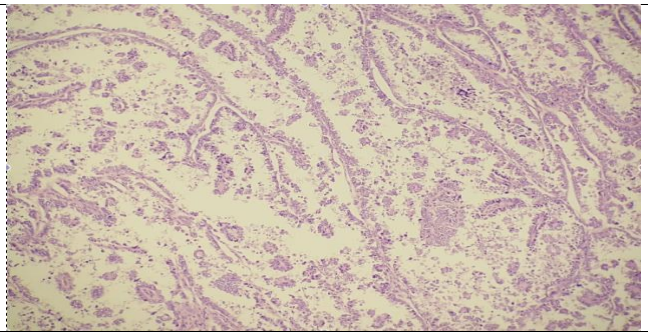


Figure-1: Clear cell carcinoma with papillary architecture (H&E, Magnification x10).

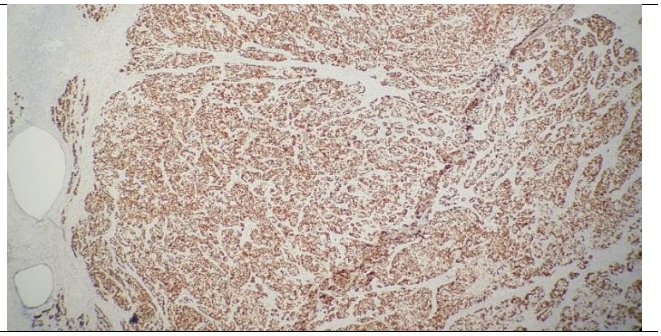


Figure-2: Napsin A Stain on Clear Cell Carcinoma. (IHC, Magnification x10).

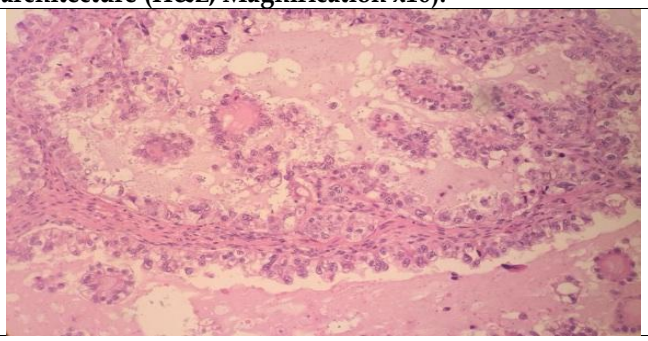


Figure-3: Clear Cell Carcinoma with hobnailing. (H&E, Magnification x40).

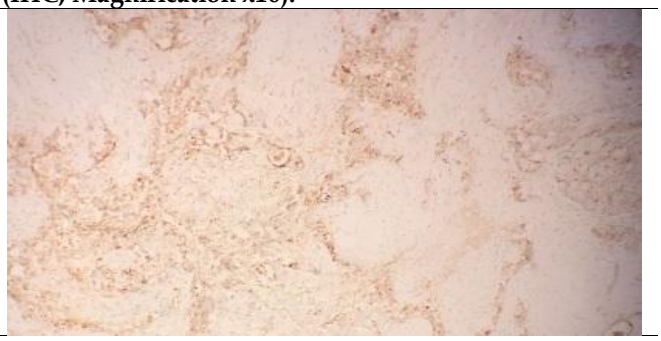


Figure-4 Napsin A on Clear Cell Carcinoma. (IHC, Magnification x10).

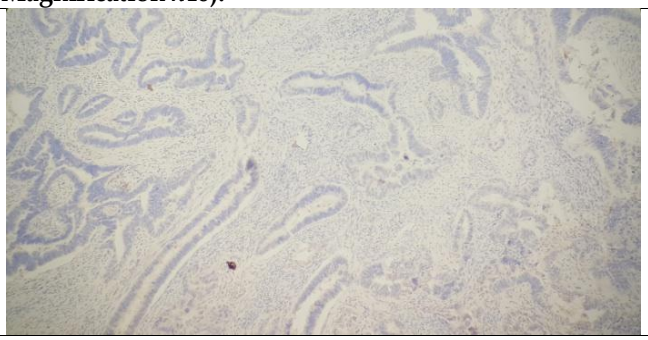


Figure-5: Napsin A on Serous Carcinoma. (IHC, Magnification x10).

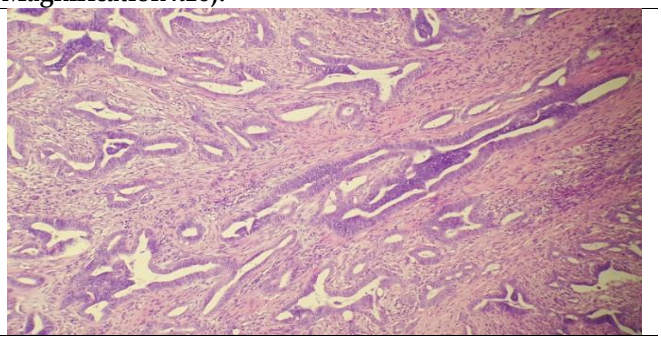


Figure-6: Serous Carcinoma with tubular and glandular architecture (H&E, Magnification x10).

and immunohistochemical expression of clear cell (fig-2, 3, 4 & 5) whereas, serous carcinoma illustrated in (fig 6 & 7), respectively. Maximum of specimens n=38 (64.4%) were received with intact capsule while others n=21 (36%) were observed to be ruptured. Among clear cell (14%) only high serous carcinoma. In addition, uterus and omentum involved in n=6 (10.1%) cases. Particularly, the cases n=38 (64.4%) were pT1 stage, while n=21 (36%) cases observed with pT2 and PT3 collectively. Data stratification according to tumor subtype is done in table.CK7

was applied and showed positive expression in all the cases. Napsin A was found positive in all the cases of clear cell carcinoma as compared to only n=1 (3%) high grade serous carcinomas and none of the mixed tumors. The relationship was considered to be significant with a *p*-value of <0.001. Detailed immunohistochemical profile of tumors is given in fig-1.

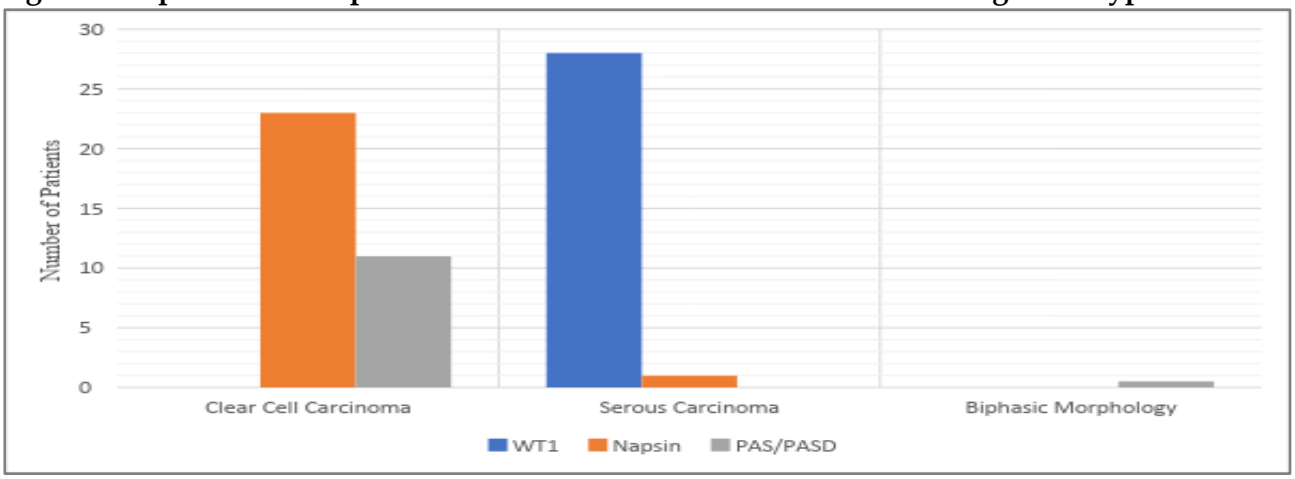
DISCUSSION

Ovarian carcinomas are among the most common tumors in females in Pakistan. According to Punjab cancer registry data[11] it is the third most common tumor in females while data from Shaukat Khanum Cancer registry[12] shows that it is the second most common case reported in adult females in the last three decades.

High grade serous carcinoma, is the most common reported tumor in ovaries. There were n=33 cases of high grade serous carcinoma in our study. The mean age of these patients was 47.15 ± 8.97 years which was less than that of *Seidman et al* which reported a median age of 65 years in their study[13]. Ayhanet.al noted in his study that most serious carcinomas presented in the advanced stage. However, half of the tumors in our study were stage-I. The most reasonable explanation for this phenomenon is that in Pakistan staging laparotomy is not carried out in all the cases[14].

Mean age of patients of clear cell carcinoma was only slightly less than that of serous carcinomas. According to Pather *et. al*[15], mean age of 56 years which is slightly more than that of the current study. However, the number of tumors limited to ovary was a lot more than in serous carcinomas. Clear cell carcinoma is commonly associated with endometriosis but in the present study only one tumor was found to have concurrent endometriosis. High grade serous carcinomas are known to behave aggressively and frequently present with metastatic disease. Therefore, it is imperative to differentiate it from other ovarian tumors. Clear cell carcinoma usually presents as a lower stage of disease and has a better prognosis. Both these tumors show morphological and immunohistochemical overlap. The most common markers used to differentiate serous carcinoma and ovarian carcinoma include WT1, P53 and HNF 1B. Using a single immunohistochemical stain can result in misdiagnosis as none of these markers are completely specific or sensitive for a single tumor. WT1 expression is seen in a large majority of serous carcinomas and is virtually absent in clear cell carcinoma. However, there is a certain proportion of tumors that do not stain for serous carcinomas. Similarly, P53 aberrant staining is

Figure-7: Expression of important immunohistochemical markers according to subtype.



Napsin was performed in all the cases in the study. Data regarding WT1 and PAS/PASD was not present in all the cases.

seen in more than 90% of serous carcinomas while almost 12% of clear cell carcinomas show aberrant expression. HNF-1B shows positive expression in about 13% of high grade serous carcinomas while it shows universal expression in all ovarian clear cell carcinomas. Although HNF-1B is highly sensitive, it suffers from low specificity as it is expressed in almost half the cases of endometrioid adenocarcinomas and all the metastatic colorectal carcinomas.Therefore, for accurate diagnosis of clear cell carcinomas a panel of immunohistochemical markers is recommended including both positive and negative markers.

Napsin A, is commonly expressed in adenocarcinomas of the lung and papillary carcinoma of the kidney. Kobel *et al* compared the expression of Napsin A among various ovarian tumors and found it to be highly specific for clear cell carcinomas[16]. The protein was expressed in more than 97% of clear cell carcinomas, while only two cases of serous carcinomas were positive for Napsin A. Five% of endometroid carcinomas also expressed the protein. *Yamashita, et al*[8] conducted a larger study with n=86 cases of clear cell carcinomas and its various mimics and found an 83% expression as compared to almost no expression in any of the other carcinomas. Only Arias-stella reaction showed complete expression. No such study has been carried out in Pakistan previously. Our results are similar to these international studies with Napsin A expression in all the cases of Clear cell carcinoma and only a single case of High grade serous carcinoma. A pathologist commonly faces a differential of clear cell carcinoma and high grade serous carcinoma. Many a time histology itself does not reveal enough clues and help of immunohistochemical markers is needed. In this scenario, we recommend that a pathologist should use a panel of immunohistochemical markers instead of relying on a single stain. However, Napsin A should be applied because it is sensitive and specific marker for diagnosis and differentiating marker for epithelial ovarian carcinomas. Furthermore, it is important to differentiate it from other high grade ovarian epithelial tumors due to its high chemoresistance. In addition, a study should be design on large sample size and checkout the expression of other immunohistochemical stains on both malignancies.

CONCLUSION

Expression of Napsin A showed a significant difference in high grade serous carcinoma and clear cell carcinoma. Therefore, Napsin A is a sensitive and specific marker for the diagnosis of ovarian clear cell carcinoma and can reliably differentiate it from high grade serous carcinoma. Current research recommends a study with large sample size to insight the expression of Napsin A and other immunohisto chemical stains.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer biology & medicine 2017; 14(1): 9.
2. Vang R, Shih IM, Kurman RJ. Ovarian low-grade and high-grade serous carcinoma: pathogenesis, clinicopathologic and molecular biologic features, and diagnostic problems. Adv Anat Pathol 2009; 16(5): 267.
3. Berns EM, Bowtell DD. The changing view of high-grade serous ovarian cancer. Cancer res 2012; 72(11): 2701-4.
4. Zannoni GF, Morassi F, Prisco MG, De Stefano I, Vellone VG, Arena V, et al. Clinicopathologic and immunohistochemical features of ovarian clear cell carcinomas in comparison with type I and type II tumors. Int J Gynecol Pathol 2012; 31(6): 507-16.
5. Anglesio MS, Carey MS, Köbel M, MacKay H, Huntsman DG. Clear cell carcinoma of the ovary: a report from the first Ovarian Clear Cell Symposium, June 24th, 2010. Gynecol oncol 2011; 121(2): 407-15.
6. Skirnisdottir I, Bjersand K, Åkerud H, Seidal T. Napsin A as a marker of clear cell ovarian carcinoma. BMC cancer 2013; 13(1): 524.
7. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. The Lancet 2014; 384(9951): 1376-88.
8. Yamashita Y, Nagasaka T, Naiki-Ito A, Sato S, Suzuki S, Toyokuni S, Ito M, Takahashi S. Napsin A is a specific marker for ovarian clear cell adenocarcinoma. Modern Pathology 2015; 28(1): 111-7.
9. Zhang C, Schmidt LA, Hatanaka K, Thomas D, Lagstein A, Myers JL. Evaluation of napsin A, TTF-1, p63, p40, and CK5/6 immunohistochemical stains in pulmonary neuroendocrine tumors. Am J Clin Pathol 2014; 142(3): 320-4.
10. Kim MY, Go H, Koh J, Lee K, Min HS, Kim MA, et al. Napsin A is a useful marker for metastatic adenocarcinomas of pulmonary origin. Histopathology 2014; 65(2): 195-206.

11. https://shaukatkhanum.org.pk/health-care-professionals-rese-archers/cancer-statistics/punjab-cancer-registry/
12. http://shaukatkhanum.org.pk/wp-content/uploads/2015/ 06/ ccrr-1994-2016.pdf
13. Seidman JD, Horkayne-Szakaly I, Cosin JA, Ryu HS, Haiba M, Boice CR, et al. Testing of two binary grading systems for FIGO stage III serous carcinoma of the ovary and peritoneum. Gynecolo oncolo 2006; 103(2): 703-8.
14. Ayhan A, Taskiran C, YIGIT CELIK N, Bozdag G, Gultekin M, Usubutun A, et al. Long term survival after paclitaxel plus platinum based combination chemotherapy for extraovarian peritoneal serous papillary carcinoma: is it different from that for ovarian serous papillary cancer?. Int J Gynecol Cancer 2006; 16(2): 484-9.
15. Pather S, Quinn MA. Clear cell cancer of the ovary - Is it chemosensitive?. Int J Gynecol Cancer 2005; 15(3): 432-7.
16. Köbel M, Rahimi K, Rambau PF, Naugler C, Le Page C, Meunier L, de Ladurantaye M, Lee S, Leung S, Goode EL, Ramus SJ. An immunohistochemical algorithm for ovarian carcinoma typing. Int J Gynecol Pathol 2016; 35(5): 7.

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