RESEARCH ARTICLE

Immuno-Positive Stained of Ki-67 from Formalin-Fixed Paraffin-Embedded Serous Cystadenoma and Serous Epithelial Ovarian Carcinoma Specimens

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Abstract:

A vast archive of biopsy and autopsy specimens processed for histological diagnosis exists in pathology laboratory worldwide has potential value for immunohistochemistry studies associated with protein identification such as Ki-67. Determination of cellular activity has been reported to be of a diagnostic with prognostic value used to estimate the number of proliferating cells in ovarian carcinoma. Ki-67 antigen was found to be a reliable diagnostic marker for estimating the Proliferative Index (PI) of a neoplastic lesion by the Mindbomb E3 ubiquitin protein ligase1 (MIB-1 antibody) using immunohistochemistry. However, there is lack of published studies about Ki-67 from serous epithelial ovarian carcinoma (SEOC) in Malaysia. Thus, the purpose of this study is to identify immuno-positive stained associated with protein expression of Ki-67 from serous cystadenoma and SEOC specimens. 76 FFPE specimens which consist of 36 serous cystadenoma and 40 SEOC samples were sectioned and subjected to immuno-histo staining using monoclonal M1B1 antibody. Immuno-positive stained for Ki-67 was analysed by the blinded consultant pathologist. The results were presented as percentage and mean of Ki-67 labelling index (LI) among the samples studied. Protein positivity of Ki-67 was detected in 49 specimens (64.5%), comprising of 9 serous cystadenoma (11.8%), 15 low-grade SEOC (19.7%), and 25 high-grade SEOC (33.0%). Mean Ki-67 LI was higher in SEOC (42.50±27.07) compared to serous cystadenoma (1.99±3.59), and the difference was statistically significant (p=0.000). This study showed that archive FFPE samples were useful to be used for immunohistochemistry study.

Keywords: Formalin-fixed paraffin-embedded samples, immunohistochemistry, Ki-67 expression, ovarian carcinoma

1. INTRODUCTION

Pelvic examination, cancer antigen 125 (CA 125) as a tumour marker, transvaginal ultrasound (TVU) and potentially multimarker panels, and bioinformatic analysis of proteome patterns are all screening and diagnostic approaches for ovarian cancer. Physical examination's sensitivity and specificity for detecting asymptomatic ovarian cancer are low, and it is not recommended as a screening tool. The sensitivity and specificity of CA 125 are both limited. Many women with ovarian cancer have elevated CA 125 levels, although only half of early ovarian malignancies produce enough CA 125 to cause a positive test (Hellstrom et al., 2003). Noncancerous gynaecological disorders, other malignancies, and other noncancerous effects can all raise CA 125 levels in the blood (Khandakar et al., 2014). TVU can identify tiny ovarian masses and can tell the difference between benign and malignant adnexal masses. Despite this, it is still ineffective at predicting which tumours are cancerous and which are caused by benign disease.

Ultrasound has demonstrated to be ineffective in detecting ovarian cancer in average-risk and high-risk women when used as an independent diagnostic (Aune et al., 2011).

Efforts to create a blood test for ovarian cancer based on measuring genes, proteins, or several marker assays that may be present in higher or lower numbers in women with ovarian cancer compared to women who do not have ovarian

cancer are still underway. Although intriguing, this study is yet experimental, and prospective validation studies will be required (Aune et al., 2011).

Ki-67 antigen, on the other hand, has been discovered to be substantially expressed in malignant ovarian tumours (Dewi et al., 2020). Tumor aggression, vascular invasion, tumour metastasis, guarded prognosis, and poor treatment response are all linked to the Ki-67 antigen. Its expression can be employed as a diagnostic and prognostic tool in the clinical therapy of ovarian cancer (Mita et al., 2004).

Therefore, this study is aimed to identify immuno-positive stained associated with protein expression of Ki-67 from formalin-fixed paraffin-embedded tissues of serous cystadenoma and SEOC specimens in Malaysia.

2. MATERIALS AND METHODS

A total of 76 FFPE specimens which consist of 36 serous cystadenoma and 40 SEOC with evidence of metastasis tissues were retrieved from the Department of Pathology, Hospital Selayang and Hospital Serdang Malaysia. This study was conducted upon approval by the UiTM Research Ethics Committee (REC/223/17) and Medical Research & Ethics Committee NMRR-17-514-34214 (IIR). All tissues were obtained as archived formalin-fixed paraffin embedded (FFPE) samples. Inclusion criteria were FFPE samples obtained from patients diagnosed with SEOC in 2013 to 2017. The FFPE tissue blocks were then cut using a microtome with 4 µm thick and deparaffinised to remove the wax.

As shown in Figure 1, the Ki-67 immunostaining was performed using monoclonal M1B1 antibody (Dako) with dilution 1:75 following the manufacturer's protocol. A minimum of 200 cells per section was counted for Ki-67 positivity and expressed as a percentage. The scoring process was done by consultant pathologist that was blinded to the clinico-pathological data. Mean Ki-67 LI was calculated by an independent sample t-test to differentiate protein expression level of Ki-67 between serous cystadenoma and SEOC specimens.

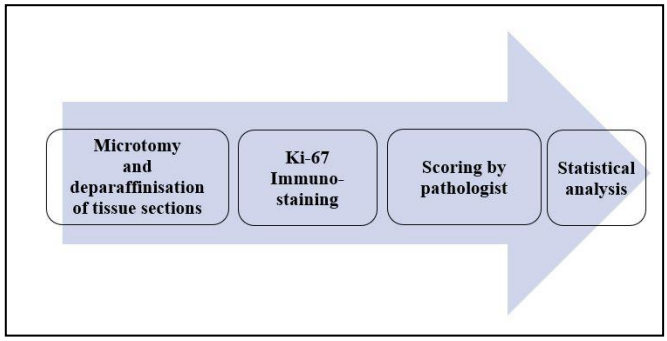

Figure 1: Workflow for determination of Ki-67 immuno-positivity study from serous cystadenoma and SEOC

3. RESULTS AND DISCUSSION

The qualitative evaluation of Ki-67 was reported according to the intensity of Ki-67 staining as presented in Table 1.

Table 1: Ki-67 Intensity Classification

Description	Ki-67 Intensity
No staining of brown colour	0
Weak, pale brown at high power objective (40x)	1
Moderate brown staining at high power objective (40x)	2
Intense dark brown staining even at low power objective (10x)	3

Figure 2 showed Ki-67 positive stained sections observed in 49 cases (64.5%) which consist of 9 cases of serous cystadenoma (11.8%), 15 cases of low-grade SEOC (19.7%) and 25 cases of high-grade SEOC (33.0%).

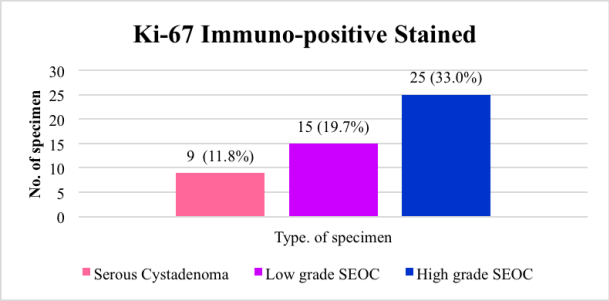

Figure 2: Overall Ki-67 immuno-positive stained in serous cystadenoma and SEOC patients

Among serous cystadenoma and SEOC group, the mean Ki-67 LI was highest in SEOC (42.50±27.07%) and least in serous cystadenoma (1.99±3.59%). There was a statistically significant difference of Ki-67 LI between control and test group (p=0.000). A study by Sardar (2018) also showed statistical difference observed between benign, borderline and malignant ovarian tumours. In this context, they reported mean Ki-67 LI of benign ovarian tumours is 3.7±2.64% and a malignant ovarian tumour is 40.15±9.3%.

In addition, a study by Giurgea et al. (2012) indicated that Ki-67 was positive in 61.53 % of malignant cases, with higher percent in advanced clinical stages while lower percent of 9.09 % in benign ovarian epithelial tumours. Moreover, Kong et al. (2016) investigated and found that FFPE tissues archived beyond two years are suboptimal for telomere analysis.

Furthermore, a study by Fujii et al. (2020) reported that FFPE would be adequate for genetic analysis, although it is desirable to store frozen specimens for the tumor tissues to be subjected to genetic analysis.

4. CONCLUSION

In conclusion, the immunohistochemistry of Ki-67 from serous cystadenoma and SEOC was successfully studied. However, the sample size did not represent the whole Malaysian population. Therefore, the future research may involve larger sample size of population. In SEOC histological grade and FIGO stage when combined with Ki-67 LI in histopathology report, it is aimed that it would help in diagnostic differentiation of subtypes, prognostication, deciding the need for adjuvant chemotherapy and in predicting survival analysis.

ACKNOWLEDGEMENTS

The authors would like to thank the Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences Universiti Teknologi MARA Cawangan Selangor, Puncak Alam campus for laboratory facilities.

This research was funded by the Institute of Research Management and Innovation UiTM [Grant number: 600-IRMI/MyRA 5/3/LESTARI (0582017)].

REFERENCES

Aune, G., Stunes, A. K., Tingulstad, S., Salvesen, O., Syversen, U., Torp, S. H. (2011). The proliferation markers Ki-67/MIB-1, phosphohistone H3, and survivin may contribute in the identification of aggressive ovarian carcinomas. Int J Clin Exp Pathol;4(5):444-53.

Dewi, I. G. A. S. M., Susraini, A. A. A. N. & Ekawati, N. P. (2020). HER2/neu and Ki-67 as prognostic factors in serous type ovarian carcinoma. *Bali Medical Journal*, 9(2):567-571.

Fujii, T., Uchiyama, T., Matsuoka, M., Myojin, T., Sugimoto, S., Nitta, Y., Okabe, F., Sugimoto, A., Sekita-Hatakeyama, Y., Morita, K., Itami, H., Hatakeyama, K., & Ohbayashi, C. (2020). Evaluation of DNA and RNA quality from archival formalin-fixed paraffin-embedded tissue for next-generation sequencing - retrospective Study in Japanese single institution. Pathology International.

Giurgea, L. N., Ungureanu, C. & Mihailovici, M. S. (2012). The immunohistochemical expression of p53 and Ki-67 in ovarian epithelial borderline tumors - correlation with clinicopathological factors. Rom J Morphol Embryol 53 (4): 967-973.Hellstrom, I., Raycraft, J., Hayden-Ledbetter, M., et al. (2003). The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res, 63:3695-3700.

Hellstrom, I., Raycraft, J., Hayden-Ledbetter, M., et al. (2003). The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res, 63:3695-3700.

Khandakar, B., Sandeep, R. Mathur., Kumar L, Kumar S, Gupta, S.D. (2014). Tissue biomarkers in prognostication of serous ovarian cancer following neoadjuvant chemotherapy. BioMed Research International. 401245, 9 pages.

Kong, P. L., Looi, L. M., Lau, T. P., & Cheah, P. L. (2016). Assessment of telomere length in archive formalin-fixed, paraffinized human tissue is confounded by chronological age and storage duration. PLOS One 11(12): e0168238.

Mita, S., Nakai, A., Maeda, S. & Takeshita, T. (2004). Prognostic significance of Ki-67 antigen immunostaining (MIB 1 monoclonal antibody) in ovarian cancer. J Nippon Med Sch. 71:384-91

Sardar, K., Singh, J. & Tirkey, S. (2018). Ki-67 immunoexpression in ovarian tumours. IOSR Journal of Dental and Medical Sciences; Volume 17, Issue 2 Ver 16; 34-37.

