UNIVERSITI TEKNOLOGI MARA

MOLECULAR ANALYSIS AND PROTEIN EXPRESSION OF KI-67 FROM FORMALIN-FIXED PARAFFIN-EMBEDDED SEROUS CYSTADENOMA AND SEROUS EPITHELIAL OVARIAN CARCINOMA SPECIMENS

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MSc

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

High mortality rates in serous epithelial ovarian carcinoma (SEOC) are explained by the fact that majority of the patients present at an advanced stage, with extensively metastatic disease within the peritoneal cavity. Therefore, it is crucial to identify the potential biomarker to detect SEOC that could be used as prognosis. This thesis aimed to provide fundamental knowledge regarding Ki-67 gene expression among SEOC patients in Malaysia. Moreover, the present study also determined gene and protein expression of Ki-67 from formalin-fixed paraffin-embedded (FFPE) tissues of SEOC to normal ovarian tissues. Molecular analysis and protein expression of Ki-67 were investigated by quantitative real-time polymerase chain reaction (RT-qPCR) and immunohistochemistry in 76 FFPE tissues, which consist of 36 normal serous cystadenoma and 40 SEOC specimens. RT-qPCR analysis using Relative Expression Software Tool (REST) 2009 showed a relative normalised expression of Ki-67 gene was up-regulated in SEOC group in comparison to serous cystadenoma by mean factor of 2.251 (standard error, S.E range 0.801 – 6.059, p=0.000). In addition, an independent sample t-test was performed to compare quantification cycle (Cq) values on Ki-67 gene expression. The finding reported that there was a statistically significant difference in Cq values for serous cystadenoma (26.4289±1.95414) and SEOC (25.4513±2.17097), where p=0.043. Furthermore, there was also a statistically significant difference in Cq values between low-grade (26.5393±1.71364) and high-grade SEOC specimens (24.7984±2.18218) where p=0.012. Moreover, chi-square test was also used to study association between International Federation of Gynaecology and Obstetrics (FIGO) stages, FFPE age of serous cystadenoma and SEOC tissue blocks with Ki-67 gene expression. The results showed no association as p>0.05, in which p=0.435, p=0.411 and p=0.312 respectively. On the other hand, 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 labelling index (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). The difference in mean Ki-67 LI between low-grade SEOC (14.13±13.71) and high-grade SEOC (59.52±16.62) was also shown to be statistically significant (p=0.000). Additionally, FIGO stages of SEOC showed significant association with mean Ki-67 LI (p=0.003). With regards to length of FFPE storage, there was no association between FFPE block tissue age and mean Ki-67 LI among SEOC specimens (p=0.407). However, there was significant association between FFPE block tissue age with mean Ki-67 LI among serous cystadenoma samples (p=0.005). In the present study, amplification and overexpression of Ki-67 suggest the aggressive behaviour of the tumour and poor clinical outcomes. Thus, Ki-67 is an exceptionally cost-effective marker to determine the growth fraction of a tumour cell population.

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