The Correlation of Expression Immunohistochemistry P53 and Matrix Metalloproteinase-9 (MMP-9) with Tumor Budding Index in Endometrial Carcinoma

Jessy Chrestella, Devi Nafilah Yuzar, Delyuzar, Lidya Imelda Laksmi, Betty
Department of Anatomical Pathology, Faculty of Medicine,Universitas Sumatera Utara, Medan, Indonesia.
devinafilah@yahoo.co.id

Abstract

Background: The Cancer Genome Atlas combines gene characteristics and identifies four groups of endometrial carcinoma, one of them is P53 mutations which are associated with poor prognosis. MMP-9 is one of the most widely observed types of MMPs to play an important role in cancer cell invasion and tumor metastasis. Tumor budding is defined as a single cell or cluster of cells consisting of < 5 cells, located at the edge of the invasive tumor front. There are relationship between tumor budding and the epithelial mesenchymal transition (EMT). The EMT process is characterized by an increase in matrix metalloproteinases that play a role in the degradation of the extracellular matrix and basement membrane, which is the process of invasion and metastasis.
Methods: This is an analytical study with cross sectional approach on 38 resection specimens from the patients diagnosed as endometrial carcinoma. The statistical analysis was performed by using Chi-square test to analyze the correlation of the correlation of expression immunohistochemistry P53 and Matrix Metalloproteinase-9 (MMP-9) with tumor budding index in endometrial carcinoma.
Results: The identification of 38 samples of endometrial carcinoma patients found that the most common age group was > 55 years, the most frequent distribution was low histopathological grading, low budding, and there was a significant correlation between the the expression of MMP-9 based on tumor budding. But no correlation between p53 expression and the tumor budding index and no correlation between p53 expression and mmp-9 in endometrial carcinoma.

Keyword : endometrial carcinoma, tumor budding, p53, matrix metalloproteinases-9

INTRODUCTION

Endometrial carcinoma is the most common type of gynecologic cancer in women in developed countries.[1] In addition, endometrial carcinoma was the fourth leading cause of death from gynecological malignancies in women worldwide in 2018.[2] According to GLOBOCAN data in 2020, there were approximately 417,000 new cases and 97,000 deaths from this malignancy.[3] Endometrial carcinoma accounts for the largest number of these cases, because less than 10% of uterine corpus cancers are sarcomas.[4]

Endometrial carcinoma is among the more common cancers in women in developed countries. The prevalence of endometrial cancer in the United States is 25.7/100,000 women per year. The lifetime risk for developing this disease is approximately 2.8% in American women. The peak ages of diagnosis are between ages 55 and 64 years with the mean age being 62 years.[5] In Indonesia, the latest study found that the prevalence of endometrial carcinoma at RSCM Jakarta reached 7.2 cases per year, where from these data, endometrial carcinoma is rarely found in the age group under 40 years. Research conducted at Haji Adam Malik General Hospital Medan in 2012-2015, the number of patients with endometrial carcinoma was found to be 48 people, of which the most patients were >55 years old.[6]

The Cancer Genome Atlas (TCGA), combines gene characteristics and identifies four groups of endometrial carcinomas. Group 1 with POLE mutations, which are associated with a good prognosis; group 2 with microsatellite instability, which is associated with an intermediate prognosis; group 3 showed low-copy-number alterations that were also associated with an intermediate prognosis; and group 4 with high-copy number alterations and P53 mutations associated with poor prognosis. The combination of microscopic appearance and molecular characteristics is the best approach to predict the prognosis of patients with endometrial carcinoma.[7,8,9]

The tumor suppressor gene P53 which encodes for the p53 protein has been established as one of the most common mutations in human tumors. This subgroup was initially defined by the high number of somatic copy-number alterations and low mutational yield but p53 IHC was later chosen as a representative method to determine tumors of this group. In endometrial cancer p53 alterations to either overexpression or missense are associated with the worst prognosis of all molecular subtypes. The group of p53 mutated includes most of the serous and mixed types, presents at higher stages, and is more commonly grade 3. Nevertheless, lower grades

and early stages are also found for p53 mutated tumors. The p53 subgroup includes 8–24% of EC. The group of EC that did not exhibit any of the previously described features was classified as p53 wildtype or no specific molecular profile (NSMP). These tumors express estrogen and progesterone receptors and predominantly present as an endometrioid subtype. The majority of EC (30–60%) exhibits this subtype.[7,8,9]

Matrix metalloproteinases are a family of zinc-dependent endopeptidases consisting of more than 20 different types.[10] MMP-9 is one of the most widely observed types of MMPs to play an important role in cancer cell invasion and tumor metastasis. Similar to MMP-2, MMP-9 belongs to the gelatinase subgroup of the MMP family.[11] Overexpression of MMP-9 is frequently observed in several malignancies. Due to its proteolytic activity in the extracellular environment, MMP-9 is involved in many biological processes. These biological processes include the proteolytic degradation of the ECM, altered interactions between cell and cell and cell and ECM, cleavage of cell surface proteins and cleavage in the extracellular environment. MMP-9 plays a role in the degradation of the basement membrane. During tumor development, supporting the basement membrane is usually an important step in tumor invasion and metastasis.[10,11,12,13]

Tumor budding is defined as a single cell or cluster of cells consisting of < 5 cells, located at the edge of the invasive tumor front. This picture is found in various types of malignancies where it gives an image in the form of finger-like projections called buds that can enter the surrounding tissue. During localized cancer growth, some of these clusters of cells detach from the main tumor body and invade the surrounding stroma. This phenomenon is considered to be the histological basis for the formation of metastases and further tumor invasion.[14-16] One of the most important aspects of the metastatic process is the relationship between tumor budding and the epithelial mesenchymal transition (EMT). EMT is a transition process of epithelial cells into mesenchymal cells, in which epithelial cells lose their intercellular adhesion and have the ability to migrate and invade which are characteristics of mesenchymal cells. The EMT process is characterized by an increase in matrix metalloproteinases that play a role in the degradation of the extracellular matrix and basement membrane, which is a prerequisite for the process of invasion and metastasis.[14-17]

MATERIALS AND METHODS

This study is an analytical study that aims to assess the correlation between the immunohistochemical expression of p53 and Matrix Metalloproteinase-9 (MMP-9) with tumor budding index in endometrial carcinoma at the Department of Anatomic Pathology Faculty of Medicine Universitas Sumatera Utara / H. Adam Malik Hospital Medan. The statistical analysis was performed by using Chi-square test to analyze correlation between the immunohistochemical expression of p53 and Matrix Metalloproteinase-9 (MMP-9) with tumor budding index in endometrial carcinoma.

P53, a DNA-binding, oligomerization domain- and transcription activation domain-containing tumor suppressor, upregulates growth arrest and apoptosis-related genes in response to stress signals, thereby influencing programmed cell death, cell differentiation and cell cycle control mechanisms.[7,8,9] P53 expression was performed immunohistochemically from the manufacturer Santa Cruz Biotechnology. P53 expression is localized to the nucleus. Assessment is categorized to be negative expression and positive expression.

MMP-9 (Matrix Metalloproteinase-9) is one type of MMP that is able to degrade various components of the extra cellular matrix that play an important role in cancer cell invasion and tumor metastasis.[10,11,12,13] MMP-9 expression was performed immunohistochemically using BZ-0898450-AP Rabbit Anti MMP-9 Polyclonal Antibody with 1:100 (40 C, overnight) dilution from the manufacturer Bioenzy. The assessment was carried out semi-quantitatively based on the intensity and percentage of tumor cells or stromal cells expressed. MMP-9 expression was characterized by brownish cytoplasm, which was assessed in 10 fields of view of microscopic preparations with an objective lens magnification of 10x. The intensity of MMP-9 is categorized to be 0: negative, 1: weak, 2: moderate, 3: strong) and the percentage is categorized to be 0: 0%, 1: 1-5%, 2: 6-75%, 3: 76-100%. The final score was determined by the combined staining score. Score <3 was defined as negative expression and score ≥ 3 as positive expression.[18,19]

Tumor budding is defined as a single cell or cluster of cells consisting of < 5 cells, located at the edge of the invasive tumor front. The tumor budding was assessed in front of an invasive tumor with a measuring field of 0.785 mm2, which corresponds to a 20x objective lens. The tumor budding will be classified as a low budding category if 0-4 buds were found, the intermediate budding category if 5-9 buds were found, and the high budding category if 10 buds were found.[20]

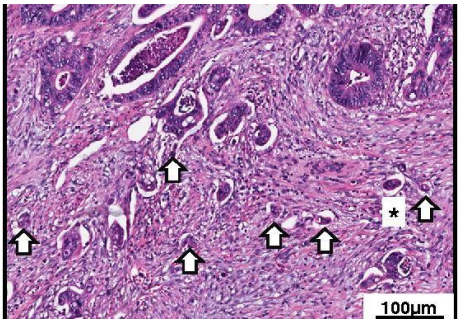

Figure 1. Tumor budding is defined as the presence of single cells or small clusters of cancer cells in the tumor stroma and is frequently found in tumorswith an invasive growth pattern and desmoplastic stromal response.[21]

RESULTS

In this study, the most common age distribution for endometrial carcinoma was the age group > 55 years, as many as 20 cases (52.6%), followed by the 45-55 year age group with 13 cases (34.2%), and the most slightly in the age group <45 years as many as 5 cases (13,2%). The frequency distribution of patients with endometrial carcinoma based on histopathological grading was mostly low grade with 26 cases (68.4%) compared to high grade with 12 cases (31.6%). The frequency distribution of endometrial carcinoma patients based on the tumor budding index was low budding as many as 22 cases (57.9%), followed by high budding as many as 9 cases (23.7%), while for intermediate budding the least as many as 7 cases (18 ,4%). The frequency distribution of endometrial carcinoma patients based on the immunohistochemical expression of MMP-9 was the most negative in 22 cases (57,9%), while the positive expression was 16 cases (42,1%). The frequency distribution of endometrial carcinoma patients based on the immunohistochemical expression of TP53, the most negative expression was 27 cases (71.1%), while the positive expression was 11 cases (28.9%).

Table 1. Frequency distribution variable of endometrial carcinoma patients

Variable	N	Percentage (%)
Age		
< 45 years	5	13,2
45-55 years	13	34,2
> 45 years	20	52,6
Grade		
Low grade	26	68,4
High grade	12	31,6
Indeks tumor budding		
Low	22	57,9
Intermediate	7	18,4
High	9	23,7
Expression MMP-9		
Negative	22	57,9
Positif	16	42,1
Expression p53		
Negative	27	71,1
Positive	11	28,9
Total	**38**	**100**

In this study 38 samples were examined, each sample was assessed for the expression of MMP-9 which was grouped based on the tumor budding index of endometrial carcinoma to examine the relationship between variables. The results of statistical test analysis showed that there was a significant correlation between the the expression of MMP-9 based on tumor budding with p value =0.021 (p<0.05).

Table 2. The correlation of expression MMP-9 with tumor budding index in endometrial carcinoma

MMP-9	Tumor Budding Index						p value*
	Low		Intermediate		High		
	N	%	N	%	N	%	
Negative	13	59,1	7	31,8	2	9,1	0,021
Positive	9	56,3	0	0	7	43,8	

*Fisher's exact test

This study also assessed the relationship between p53 expression and the tumor budding index of endometrial carcinoma. Based on the hypothesis test, there was no correlation between p53 expression and the tumor budding index of endometrial carcinoma with a p value =0.468 (p>0.05).

Table 3. The correlation of expression p53 with tumor budding index in endometrial carcinoma

P53	Tumor Budding Index						p value*
	Low		Intermediate		High		
	N	%	N	%	N	%	
Negative	14	51,9	6	22,2	7	25,9	0,468
Positive	8	72,7	1	9,1	2	18,2	

*Chi square test

In this study, the relationship between p53 expression and mmp-9 expression was also assessed. Based on the hypothesis test, there was no correlation between between p53 expression and mmp-9 in endometrial carcinoma with a p value =0,086 (p>0.05).

Table 4. The correlation of expression p53 with mmp-9 in endometrial carcinoma

P53	MMP-9				p value*
	Negative		Positive		
	N	%	N	%	
Negative	18	66,7	9	33,3	0,086
Positive	4	36,4	7	63,6	

*Chi square test

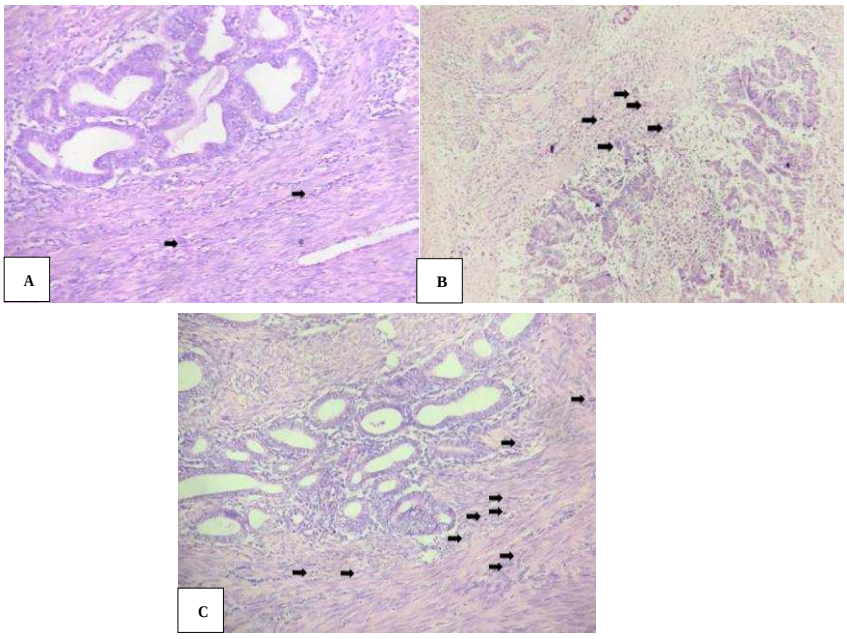

Figure 2. Tumor budding index. A. Low budding (H&E 200x), B. Intermediate budding (H&E 200x), C. High budding (H&E 2 00x).

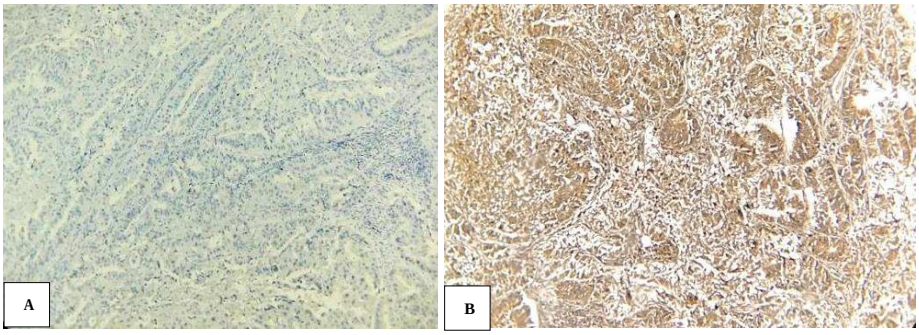

Figure 3. Expression of MMP-9. A. Negative (H&E 100x), B. Positive (H&E 100x)

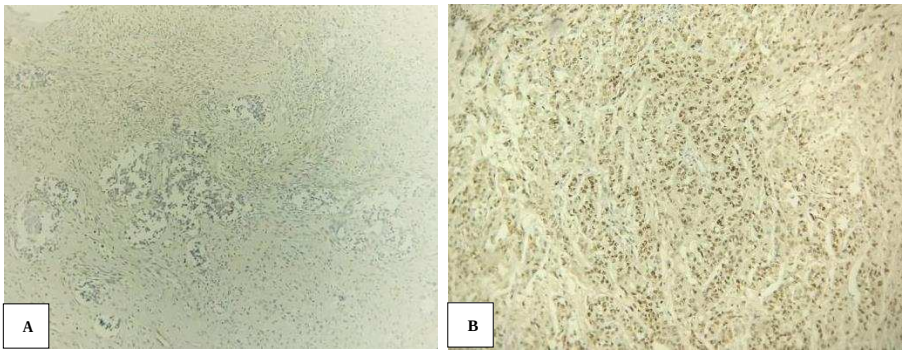

Figure 4. Expression p53. A. Negative (H&E 100x), B. Positive (H&E 100x)

DISCUSSION

In this study, the age of patients with endometrial carcinoma was mostly found in the group > 55 years, as many as 20 cases (52.6%), followed by the 45-55 year age group with 13 cases (34.2%), and the most slightly in the age group <45 years as many as 5 cases (13,2%). This result is almost the same as the research by Sofyan, where the most patients were aged >51-60 years (47,37%), followed by patients aged > 60 years (21,05%) and 40-50 years (21.05%), and the least are patients aged < 40 years (10,53%).[22] Research at Sanglah Hospital, Denpasar for the period August 2012-July 2014, also found that the most endometrial carcinoma patients were found in the 51-60 year age group.[23] Research at Haji Adam Malik Hospital Medan in 2012-2015, the number of patients with endometrial carcinoma was found as many as 48 people, where the most patients were aged > 55 years (45.8%), followed by patients aged 45-55 years (31.3 %) and the least are patients with age <45 years (22.9%).[24] This is consistent with the literature that the risk of endometrial carcinoma increases in women with advancing age, and usually occurs in postmenopausal women with high concentrations of total estrogen. The use of hormone replacement therapy in postmenopausal women containing estrogen without a combination with progesterone also increases the risk of endometrial carcinoma. Older menopause age and earlier age of menarche are also a risk due to prolonged exposure to the hormone estrogen.[25,26]

The frequency distribution of patients with endometrial carcinoma based on histopathological grading in this study was low grade, which was 26 cases (68.4%) compared to high grade with 12 cases (31.6%). This result is the same as Koyuncuoglu's study which also obtained similar results where the most grading was found in grade 2 as many as 37.9% cases, followed by grade 1 in 31.6% cases, and grade 3 in 30.5% cases.[16] Research by Zhang et al involving 1434 cases of endometrial carcinoma also found the highest results were found in cases which were a combination of grade 1 and 2, namely 1212 (84.52%) cases, while grade 3 were 222 (15.48%) cases.[27] This is in accordance with the literature which states that endometrioid carcinoma is the most common subtype of endometrial carcinoma, which is about 83% of cases where about 80-90% of them are low grade.[25,28,29]

In this study, the frequency distribution of patients with endometrial carcinoma based on the tumor budding index, the highest was low budding as many as 22 cases (57.9%), followed by high budding as many as 9 cases (23.7%), while for intermediate budding the least was as much as 7 cases (18.4%). This is same result with a research by Koyuncuoglu found that the most cases of endometrial carcinoma were found in the low grade category of tumor budding, namely 76.8% of cases compared to high grade of 23.2% of cases[16]. The study of Klutz et al found that tumor budding was positive in 72% of endometrioid carcinoma cases and 67% of cases of non-endometrioid carcinoma compared with negative budding tumors.[30] Tumor budding is a

phenomenon of cancer cells in the process of invasion. This feature is described as a sign of cancer cell motility and as an early step in the metastatic process. One of the most important aspects of the metastatic process is the relationship between tumor budding and the epithelial mesenchymal transition (EMT). EMT is a transition process of epithelial cells into mesenchymal cells, in which epithelial cells lose their intercellular adhesion and have the ability to migrate and invade which are characteristics of mesenchymal cells.[16]

The frequency distribution of endometrial carcinoma patients based on the immunohistochemical expression of MMP-9 in this study was the most negative in 22 cases (57,9%), while the positive expression was 16 cases (42,1%). MMP-9 is involved in several biological processes, namely proteolytic degradation of the Extra Cellular Matrix (ECM), changes in interactions between cells and cells as well as cells and ECM, cell surface protein cleavage and protein cleavage in the extracellular environment. MMP-9 plays a role in the degradation of the basement membrane, because the basement membrane contains collagen, including Type IV collagen, which can be degraded by MMP-9. During tumor development, destruction of the basement membrane is usually an important step in favor of tumor invasion and metastasis.[10-13]

In this study, the frequency distribution of endometrial carcinoma patients based on the immunohistochemical expression of P53, the most negative expression was 27 cases (71.1%), while the positive expression was 11 cases (28.9%). p53 plays an important role in the regulating of cell proliferation, DNA repair, apoptosis, genomic stability, senescence, and metabolic homeostasis. p53 protein activated by several signals, such as DNA damage, hypoxia, oncogene expression, ribonucleotide depletion, and osmotic stress, acts mainly as a transcriptional factor. When DNA is damaged, p53 induces the expression of p21. p21 is a cyclin-dependent kinase (CDK) inhibitor that suppresses cyclin-CDK complexes, resulting in cell cycle arrest in the G1 phase. G1 arrest can allow DNA repair before replication at S1. If the cells cannot repair the DNA damage, p53 induces apoptosis by activating apoptosis signal genes, such as BAX, PUMA, Noxa, and PERP. Loss of p53 function allows abnormal cell proliferation and is closely associated with carcinogenesis. Dysfunction of p53 has been observed in many malignant tumors.[31] P53 immunohistochemistry has evolved into an accurate surrogate reflecting the underlying p53 mutation status of a tumor, and has utility in the diagnostic workup of endometrial carcinomas. The mutational status of p53 is the single most important molecular factor, which predicts prognosis in endometrial carcinomas, with the presence of a p53 mutation being associated with an unfavorable outcome.[32]

This study aims to analyze the correlation between expression of MMP-9 and tumor budding index of endometrial cancer. After analyzing statistical tests with Chi-Square test, there was a significant correlation between the the expression of MMP-9 based on tumor budding with p value =0.021 ($p<0.05$). There are very few studies on the relationship between MMP-9 expression and tumor budding in malignancies. Several studies have been conducted on colorectal cancer and breast cancer. From these studies, it was found that high MMP-9 expression was associated with high tumor budding grades.[33-35] Tumor budding is associated with an epithelial mesenchymal transition (EMT). EMT is a transition process of epithelial cells into mesenchymal cells, in which epithelial cells lose their intercellular adhesion and have the ability to migrate and invade which are characteristics of mesenchymal cells. In addition to being characterized by a loss of E-cadherin expression, the EMT process is also characterized by an increase in matrix metalloproteinases that play a role in degrading the extracellular matrix and basement membrane, which are prerequisites for invasion and metastasis.[17-20]

This study also assessed the relationship between p53 expression and the tumor budding index of endometrial carcinoma. Based on the hypothesis test, there was no correlation between p53 expression and the tumor budding index of endometrial carcinoma with a p value =0.468 ($p>0.05$). Research by Rau et al. showed that, in a cohort of 255 ECs, the presence of tumor budding in 26.3% of the cases was independently associated with a worse prognosis. However, when the TCGA classification was used, tumor budding lost its prognostic value. Tumor budding differs significantly in its expression levels between TCGA subgroups, but cases with tumor budding were found from POLEmut, MMRdef, NSMP, and P53abn EMCAs.[36] In research of Coada et al, tumor budding was found in 35% of cases. When stratifying EC patients based on the TCGA scheme, the POLE and MMRd groups showed the highest numbers of cases with tumor budding.[37]

In this study, the relationship between p53 expression and mmp-9 expression was also assessed. Based on the hypothesis test, there was no correlation between between p53 expression and mmp-9 in endometrial carcinoma with a p value =0,086 ($p>0.05$). There is no study that has analyzed the relationship between p53 expression and mmp-9 in endometrial carcinoma. In endometrial cancer p53 alterations to either overexpression or missense are associated with the worst prognosis of all molecular subtypes.[7,8] MMP-9 expression was found to be associated with the development of gynecological malignancies, including endometrial carcinoma. MMP-9 facilitates the invasion and degradation of the ECM which is associated with cancer spread and metastasis. Research conducted to assess the expression of MMP-9 with endometrial carcinoma is still limited and still controversial. Since MMP-9 is an important factor for several cancers and several other MMP-9-associated diseases, therapeutic targeting of MMP-9 is of great importance.[38-40]

CONCLUSION

After conducting research on 38 samples of endometrial cancer it found that the most common age group was > 55 years (52,6%), the most frequent distribution was low histopathological grading (68,4%) low budding (57,9%), and there was a significant correlation between the the expression of MMP-9 based on tumor budding with p value =0.021. But no correlation between p53 expression and the tumor budding index with a p value =0.468 and no correlation between between p53 expression and mmp-9 in endometrial carcinoma with a p value =0,086.

ACKNOWLEDGEMENTS

We acknowledge to the Department of anatomical pathology, Universitas Sumatera Utara and H.Adam Malik General Hospital, Medan, Indonesia for their help and cooperation.

ETHICAL APPROVAL

Health Research Ethical Committee. Universitas Sumatera Utara, Medan, Indonesia approved thisstudy.

AUTHORS DETAILS

dr. Jessy Chrestella, M.Ked (PA), Sp.PA, Lecture and staff anatomical Pathology Departement, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

dr. Devi Nafilah Yuzar, M. Ked (PA), resident of Anatomical Pathology Departement, Faculty ofMedicine, Universitas Sumatera Utara, Medan, Indonesia.

Dr. dr. Delyuzar, M.Ked (PA), Sp.PA (K), Lecture and staff anatomical Pathology Departement, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Dr. dr. Lidya Imelda Laksmi, M.Ked (PA), Sp.PA (K), Lecture and staff anatomical Pathology Departement, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Dr. dr. Betty, M.Ked (PA), Sp.PA (K), Lecture and staff anatomical Pathology Departement, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Corresponding Author-dr. Devi Nafilah Yuzar, resident of Anatomical Pathology Departement, Faculty of Medicine, Universitas Sumatera Utara, Jl. Universitas no.1 Gedung Abdul Hakim, Keruntung, Medan, Indonesia.
Emai ID: devinafilah@yahoo.co.id

REFERENCES

1. Faizan U, Muppidi V. Uterine Cancer. [Updated 2021 Sep 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562313/
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. (2018) 68:394–424. doi: 10.3322/caac.21492
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.
4. Faizan U, Muppidi V. Uterine Cancer. [Updated 2021 Sep 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562313/
5. Mahdy H, Casey MJ, Crotzer D. Endometrial Cancer. [Updated 2021 Aug 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK525981/
6. Christian J. Karakteristik Penderita Kanker Endometrium di RSUP. H. Adam Malik Medan Tahun 2012-2015. Skripsi. Universitas Sumatera Utara. 2016
7. Matias GX, Longacre TA, McCluggage WG, Nucci MR, Olivia E. Tumours of the uterine corpus. In: Kurman RJ, Carcangiu ML, Herington CS, Young RH, editors. WHO classification of tumors of female genital tumours. 5th ed. Lyon: IARC, 2020.pp.246-6
8. Alexa M, Hasenburg A, Battista MJ. The TCGA Molecular Classification of Endometrial Cancer and Its Possible Impact on Adjuvant Treatment Decisions. Cancers (Basel). 2021;13(6):1478. Published 2021 Mar 23. doi:10.3390/cancers13061478

9. Marnitz S, Walter T, Schömig-Markiefka B, Engler T, Kommoss S, Brucker SY. A Modern Approach to Endometrial Carcinoma: Will Molecular Classification Improve Precision Medicine in the Future?. Cancers (Basel). 2020;12(9):2577. Published 2020 Sep 10. doi:10.3390/cancers12092577
10. Huang H. Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. Sensors (Basel). 2018;18(10):3249. Published 2018 Sep 27. doi:10.3390/s18103249
11. Pego ER, Fernandez I, Nunez MJ. Molecular basis of the effect of MMP-9 on the prostate bone metastasis: A review. Urol. Oncol. 2018;36:272–282. doi: 10.1016/j.urolonc.2018.03.009. [PubMed]
12. Zhang Y, Chen Q. Relationship between matrix metalloproteinases and the occurrence and development of ovarian cancer. Braz. J. Med. Biol. Res. 2017;50:e6104. doi: 10.1590/1414-431x20176104
13. Li H, Qiu Z, Li F, Wang C. The relationship between MMP-2 and MMP-9 expression levels with breast cancer incidence and prognosis. Oncology Letters 14.5 (2017): 5865-5870.
14. Singh N, Hirschowitz L, Zaino R, Alvarado CI, Duggan MA, Ali R, et al. Pathologic Prognostic Factors in Endometrial Carcinoma (Other Than Tumor Type and Grade). Int J Gynecol Pathol. 2019;38 Suppl 1(Iss 1 Suppl 1):S93-S113. doi:10.1097/PGP.0000000000000524
15. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2018. CA Cancer J Clin 68: 7-30, 2018
16. Koyuncuoglu M, Okyay E, Saatli B, Olgan S, Akin M, Saygili U. Tumor budding and E-Cadherin expression in endometrial carcinoma: are they prognostic factors in endometrial cancer?. Gynecol Oncol. 2012;125(1):208-213. doi:10.1016/j.ygyno.2011.12.433
17. Lugli A, Zlobec I, Berger MD, Kirsch R, Nagtegaal ID. Tumour budding in solid cancers. Nat Rev Clin Oncol. 2021 Feb;18(2):101-115. doi: 10.1038/s41571-020-0422-y. Epub 2020 Sep 8. PMID: 32901132
18. Yang B, Tang F, Zhang B, Zhao Y, Feng J, Rao Z. Matrix metalloproteinase-9 overexpression is closely related to poor prognosis in patients with colon cancer. World J Surg Oncol. 2014;12:24. Published 2014 Jan 29. doi:10.1186/1477-7819-12-24
19. Ashoor FZ. Matrix Metalloproteinase–9 Expressions Associated With Malignant Colorectal Carcinoma , 2017;16: 74-78
20. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol. 2017 Sep;30(9):1299-1311. doi: 10.1038/modpathol.2017.46. Epub 2017 May 26. PMID: 28548122.
21. Koelzer, Viktor Hendrik et al. "Tumor budding in colorectal cancer--ready for diagnostic practice?" Human pathology 47 1 (2016): 4-19 .
22. Sofyan N, Sudiana IK, Askandar B. Profile of endometrial cancer patients in the third referral hospital in Surabaya based on Known Risk Factors. BIOMOLECULAR AND HEALTH SCIENCE JOURNAL 2020 OCTOBER, VOL 03 (02).
23. Dewi PPP, Budiana ING. Profil Pasien Kanker Endometrium di RSUP Sanglah Denpasar Periode Agustus 2012 – Juli 2014. Medika. 2017; 6 (8): 1-7
24. Christian. J. Karakteristik Penderita Kanker Endometrium di RSUP. H. Adam Malik Medan Tahun 2012-2015. Skripsi. Universitas Sumatera Utara. 2016
25. Matias GX, Longacre TA, McCluggage WG, Nucci MR, Olivia E. Tumours of the uterine corpus. In: Kurman RJ, Carcangiu ML, Herington CS, Young RH, editors. WHO classification of tumors of female genital tumours. 5th ed. Lyon: IARC, 2020.pp.246-6
26. Mutter GL, Guiu XM, Lax SF. Endometrial Adenocarcinoma. In Robboy SJ, Mutter GL, Prat J, Bentley RC, Russel P, Anderson MC. Robboy's Pathology of the Female Reproductive Tract 2nd Ed. Churchill Livingstone Elsevier. 2009; p. 393 – 422.
27. Zhang Z, Yang B, Zhang W, Gao X, Zhao C, Zhang X, et al. Clinicopathological characteristics and survival outcomes of patients with coexistence of adenomyosis and endometrial carcinoma. Int J Clin Exp Pathol. 2018;11(2):956-962. Published 2018 Feb 1.
28. Mahdy H, Casey MJ, Crotzer D. Endometrial Cancer. [Updated 2021 Aug 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK525981/
29. Casey MJ, Summers GK, Crotzer D. Endometrial Cancer. [Updated 2020 Jul 13]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK525981/
30. Kluz T, Łoziński T, Czekierdowska S, Stachowicz N, Gurynowicz G, Chróściel M, et al. Tumor budding index and microvessel density assessment in patients with endometrial cancer: A pilot study. Oncol Lett. 2020;20(3):2701-2710. doi:10.3892/ol.2020.11811
31. Nakamura M, Obata T, Daikoku T, Fujiwara H. The Association and Significance of p53 in Gynecologic Cancers: The Potential of Targeted Therapy. Int J Mol Sci. 2019;20(21):5482. Published 2019 Nov 4. doi:10.3390/ijms20215482
32. Köbel M, Ronnett BM, Singh N, Soslow RA, Gilks CB, McCluggage WG. Interpretation of P53 Immunohistochemistry in Endometrial Carcinomas: Toward Increased Reproducibility. Int J Gynecol Pathol. 2019;38 Suppl 1(Iss 1 Suppl 1):S123-S131. doi:10.1097/PGP.0000000000000488
33. Guzińska-Ustymowicz K. MMP-9 and cathepsin B expression in tumor budding as an indicator of a more aggressive phenotype of colorectal cancer (CRC). Anticancer Res. 2006 Mar-Apr;26(2B):1589-94. PMID: 16619576
34. Sriwidyani N, Manuaba, Ida, Alit I, Astawa, Nyoman. Tumor Budding in Breast Carcinoma: Relation to E-Cadherin, MMP-9 Expression, and Metastasis Risk. 2016. Bali Medical Journal. 5. 150. 10.15562/bmj.v5i3.335.
35. González LO, Eiro N, Fraile M, Sánchez R, Andicoechea A, Fernández-Francos S, et al. Joint Tumor Bud-MMP/TIMP Count at the Invasive Front Improves the Prognostic Evaluation of Invasive Breast Carcinoma. Biomedicines. 2021 Feb 16;9(2):196. doi: 10.3390/biomedicines9020196. PMID: 33669393; PMCID: PMC7920253.
36. Rau, T.T., Bettschen, E., Büchi, C. et al. Prognostic impact of tumor budding in endometrial carcinoma within distinct molecular subgroups. Mod Pathol **34,** 222–232 (2021). https://doi.org/10.1038/s41379-020-0626-9
37. Coada CA, Dondi G, Ravegnini G, De Leo A, Santini D, De Crescenzo E, Tesei M, Bovicelli A, Giunchi S, Dormi A, Di Stanislao M, Morganti AG, De Biase D, De Iaco P, Perrone AM. Classification Systems of Endometrial Cancer: A Comparative Study about Old and New. Diagnostics (Basel). 2021 Dec 24;12(1):33. doi: 10.3390/diagnostics12010033. PMID: 35054199; PMCID: PMC8774791.
38. Michalczyk K, Cymbaluk-Płoska A. Metalloproteinases in Endometrial Cancer-Are They Worth Measuring? Int J Mol Sci. 2021 Nov 19;22(22):12472. doi: 10.3390/ijms222212472. PMID: 34830354; PMCID: PMC8624741
39. Yu F, Jiang Q, Zhou Y, Yang Z, Yu X, Wang H, et al. Abnormal expression of matrix metalloproteinase-9 (MMP9) correlates with clinical course in Chinese patients with endometrial cancer. Dis Markers. 2012;32(5):321-7. doi: 10.3233/DMA-2011-0886. PMID: 22674412; PMCID: PMC3826421.
40. Grybos A, Bar J. The relationships between the immunoexpression of KAI1, MMP-2, MMP-9 and steroid receptors expression in endometrial cancer. Folia Histochem Cytobiol. 2014;52(3):187-94. doi: 10.5603/FHC.2014.0022. PMID: 25308734