

Association of Anaplastic Lymphoma Kinase (ALK) Immunohistochemical Expression with Endometrioid Endometrial Carcinoma (EEC) Histopathological Grading

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Abstract- EEC is a primary epithelial malignant tumor in the endometrium, a glandular neoplasm with an acinar, papillary or partially solid configuration. Histopathological grading is made based on the degree of structural differentiation and cell atypia. The ALK gene is oncogenic in three ways; 1) forming a fusion gene with one of several other genes 2) obtaining additional copies of the gene 3) mutation of the actual DNA code for the gene itself. ALK rearrangement detection was carried out in 3 ways, namely Real-Time Polymerase Chain Reaction (RT-PCR), Fluorescence In Situ Hybridization (FISH), Immunohistochemistry (IHC). EEC molecular examination was found to contain chromosome 2 inversion which resulted in the EML4-ALK gene fusion. Confirmation of immunohistochemical examination showed excess ALK in EEC even though only part of the tumor. This study used a sample of endometrial tissue to assess the relationship between ALK immunohistochemical expression and EEC histopathological grade. Paraffin blocks from 31 EEC patients were sliced and then stained with ALK immunohistochemistry was used to study ALK immunohistochemical expression. The basic characteristics of the sample are obtained through Medical Records. The relationship between ALK expression and EEC histopathological grading was analyzed using SPSS version 22. The analysis showed that there was no significant relationship between ALK and EEC histopathological grading ($p > 0.05$).

Index Terms- Endometrioid Endometrial Carcinoma (EEC), Grading histopathology, Anaplastic Lymphoma Kinase (ALK)

I. INTRODUCTION

Endometrial carcinoma is the most common carcinoma of the female reproductive tract in developed countries and is the third most common cause of death in carcinoma in women. In 2012, endometrial carcinoma occurred in 320,000 women and caused 76,000 deaths worldwide.¹ In developed countries, 75% of cases of endometrial carcinoma occur in patients around 60 years of age during the post-menopausal period, so the most common symptom is post-bleeding menopause. The relationship between immunohistochemical expression of Anaplastic

Lymphoma Kinase (ALK) and grading of endometrioid endometrial carcinoma (EEC) in the human population is still limited. In Indonesia, the prevalence of endometrial carcinoma at Cipto Mangunkusumo Hospital (RSCM) Jakarta reaches 7.2 cases per year. Patients tended to be younger, as much as 63.9% at ≥ 50 years of age and as much as 12.5% at ≤ 40 years of age. The most common types based on histopathological type are Endometrioid Endometrial Carcinoma (EEC) around 75-80%, serous papillary carcinoma 5-10% and clear cell carcinoma 3-5%. Serous papillary carcinoma and clear cell carcinoma are aggressive types.²⁻⁵

Craig et al (2018) stated that molecular EEC examinations were found to contain inversion on chromosome 2 which resulted in the fusion of the Echinoderm Microtubule associated protein Like 4-Anaplastic Lymphoma Kinase (EML4-ALK) gene. Immunohistochemical examination confirmed that ALK was fused in part of the tumor. Additional genomic characterization of tumor areas deprived of ALK expression by immunohistochemistry closely matched the genomic profile of the ALK-positive section, showing the same pattern of copy-number variations and mutations in TP53 and KDM5C, confirming that EML4-ALK rearrangements have occurred as a subclonal process. EML4-ALK fusion is a guide in 2% -5% of non-small-cell lung cancers for which crizotinib is the approved therapeutic target for this disease. EML4-ALK rearrangement has not previously been reported in endometrial cancer.⁶

EEC malignancy differentiation according to the World Health Organization (WHO) is made based on cell atypia and structural histopathology. The grading is classified based on the degree of differentiation of the EEC as seen from the solid growth pattern of non-squamous or non-morula. Core grades are determined by variations in core size and shape, core size and chromatin distribution.⁷

II. MATERIAL AND METHODS

Sample selection

This study was conducted cross-sectional in the Department of Anatomical Pathology, H. Adam Malik General Hospital, Medan and included 31 EEC cases, which aimed to assess the

relationship of Anaplastic Lymphoma Kinase (ALK) immunohistochemical expression with histopathological grading of Endometrioid Endometrial Carcinoma (EEC). All samples were obtained by surgical procedure. Detailed clinical data are obtained from medical records or pathology archives covering age. The histopathologic grading was determined independently by the investigators by means of hematoxylin and eosin slide examinations.

Anaplastic Lymphoma Kinase (ALK)

ALK is an enzyme known in humans as the Tyrosine Kinase Receptor ALK or CD 246. The human ALK / LTK receptor ligand was identified more recently, in 2014; FAM150A (AUG β) and FAM150B (AUG α) are two small peptides secreted to activate ALK signaling. Anaplastic Lymphoma Kinase (ALK) was originally discovered in 1994 in Anaplastic Large Cell Lymphoma (ALCL) so that the term ALK is taken from this name, where a translocation process occurs between chromosomes 5q35 which was not previously identified by the Tyrosine Kinase protein gene located on chromosome 2p23.⁸

Immunohistochemistry Anaplastic Lymphoma Kinase (ALK)

ALK antibodies stain tumors in the cytoplasm. 9 ALK expression is determined by assessing clinical scores based on the percentage of cells expressed on immunohistochemistry (scale 0-3) and the intensity of the staining (scale 0-3), where 0: if not stained, 1: weakly stained, 2: colored medium, 3: strong colored. The percentage of cells expressed as 0: <10%, 1: 11–40%, 2: 41–70%, 3: \geq 71%. The two scores are then added together with the results: Negative (total score 0-3), Positive (total score \geq 4).¹⁰

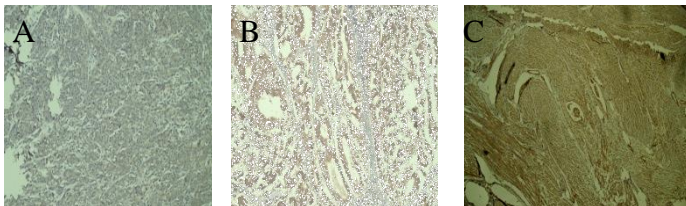


Figure 2. Expression of ALK Intensity A. Weak, B. Moderate, C. Strong

Grading

Histopathological grading is made based on the degree of structural differentiation and cell atypia. Grading is seen from the solid growth pattern of non-squamous or non-morula as follows: G-I (5% or less), G-II (6-50%), G-III (> 50%). Core grades are determined by variations in the size and shape of the core, distribution of chromatin, and size of nucleoli. Grade I core is oval, slightly enlarged, and has evenly distributed chromatin. Grade III nuclei are markedly enlarged and pleomorphic, irregular coarse chromatin and eosinophilic and prominent nucleoli. Grade-II core overview between grade-I and grade-III. Mitotic activity generally increases with increasing core grade.^{8,11-15}

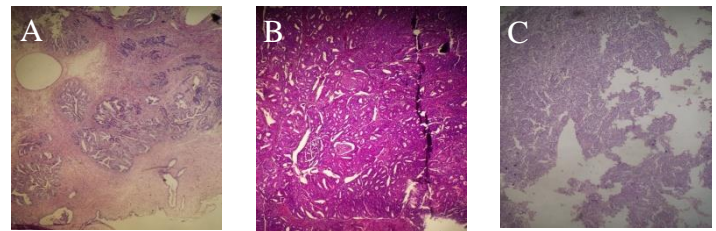


Figure 1. EEC A. Grade I, B. Grade II, C. Grade III

Statistical analysis

Statistical analyzes were performed using the software package SPSS version 22.0 (SPSS Inc., Chicago) with 95% confidence intervals and Microsoft Excel 2010. Categorical variables are presented as frequencies and percentages. Mann-Whitney U test was applied to find the relationship between ALK immunohistochemical expression and EEC histopathological grading. A p-value > 0.05 was considered insignificant

III. RESULT

The mean age for Endometrioid Endometrial Carcinoma (EEC) patients was 53.4 (\pm 14.1) with the youngest age group 21-30 being 1 case (3.2%) and the oldest age being the age group > 80 years with a sample size of 1 case (3.2%), and the age of most patients was in the age group of 51-60 years with a sample size of 11 cases (35.5%). The characteristics by age of EEC patients are summarized in Table 1.

Table 1. Characteristics of EEC patients based on age

| Age | Number of case (n) | Percentage (%) |
|---|--------------------|----------------|
| Age, mean \pm SB, years 53.4 \pm 14.1 | | |
| 21 – 30 | 1 | 3,2 |
| 31 – 40 | 4 | 12,9 |
| 41 – 50 | 7 | 22,6 |
| 51 – 60 | 11 | 35,5 |
| 61 – 70 | 3 | 9,7 |
| 71 – 80 | 4 | 12,9 |
| >80 | 1 | 3,2 |
| total | 31 | 100,0 |

The frequency distribution of EEC patients based on histopathological grading obtained data on the frequency of EEC patients with grade I histopathological grading as many as 14 people (45.2%), Grade II as many as 8 people (25.8%), and Grade III was 9 people (29.0%) listed in table 2

Table 2. Grading of EEC patients histopathology.

| Grading | Total (n) | Percentage (%) |
|-----------|-----------|----------------|
| Grade I | 14 | 45,2 |
| Grade II | 8 | 25,8 |
| Grade III | 9 | 29,0 |
| total | 31 | 100 |

Analysis of the immunohistochemical relationship of ALK with EEC histopathological grading obtained data, namely Grade I,

there were 4 cases (30.8%) showing negative expressions while the remaining 10 cases (55.2%) expressed positive. EEC Grade II, found 3 cases (23.1%) showed a negative expression and only 4 cases (27.8%) showed a positive expression. For EEC grade 3 negative ALK expression was found more than 6 cases (46.1%) than positive expression in 3 cases (16.6%). listed in table 3

Table 3. Analysis of the relationship between ALK and EEC grading

| No. | ALK expression | Grading ALK | | | | | | p-value* |
|-----|----------------|-------------|------|---------|------|---------|------|----------|
| | | Grade 1 | | Grade 2 | | Grade 3 | | |
| | | n | % | n | % | n | % | |
| 1. | Negative | 4 | 30,8 | 3 | 23,1 | 6 | 46,1 | 0.0895 |
| 2. | Positive | 10 | 55,2 | 4 | 27,8 | 3 | 16,6 | |

Based on the WHO classification, EEC consists of grade I, grade II and grade III. And BMI consists of 4 categories of underweight, normoweight, overweight and obesity. The frequency distribution of all EEC patients based on BMI and histopathological grading can be seen in table 4.

IV. DISCUSSION

In this study, 31 samples of EEC sufferers were found in the age range 51-60 years (35.5%). This condition is in accordance with the research of Sofian A (2010) where the age of EEC sufferers is ≥ 50 years of age.³ Meanwhile, in the research of Nevadunsky NS (2014), the results showed that the mean age of EEC patients was 67.1 years with a standard deviation of ± 11.9 years.¹⁶ From this study it was also found that the highest number of EEC sufferers was grade I as many as 14 people (45.2%). This is in accordance with the research of Nevadunsky NS (2014) which also states that the highest number of EEC sufferers is grade I as many as 380 people (64.3%)¹⁶. Meanwhile in O.G. Trifanescu was found to have the highest number of EEC sufferers in histopathology grade III.¹⁷

ALK is an enzyme known in humans as receptor Tyrosine Kinase ALK or CD246.¹⁸ Chromosomal rearrangements that produce gene fusion in general are the cause of ALK gene distortion in cancer, which has the potential to rapidly increase oncogens. The ALK gene can be oncogenic in three ways; 1) forming a fusion gene with one of several other genes 2) obtaining additional copies of the gene 3) mutation of the actual DNA code for the gene itself. This situation has been proven by the presence of NPM1-ALK fusion and EML4-ALK fusion in Non-Small-Cell Lung Cancer (NSCLC). Increasing the number of point mutations that activate protein kinases will also activate oncogenes in ALK.¹⁹⁻²²

The Mann-Whitney U test shows a statistical number of 0.0895. Although statistically there is not enough evidence to say that there is a relationship between ALK expression and EEC grading, from the percentage of Grade I EEC that are positive with ALK there are 10 cases (55.2%) so that the researcher believes that there is something that needs to be explored more

deeply. again and can be used as a reference or a basis for further research.

V. CONCLUSION

There was no significant relationship between ALK immunohistochemical expression and EEC histopathological grade.

COMPETING INTERESTS

The author has no financial interests relevant to the product or company described in this article.

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ETHICAL APPROVAL

Health Research Ethical Committee, University of Sumatera Utara, Medan, Indonesia approved this study.

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