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MEAN AgNOR COUNTS IN NON-ATYPICAL HYPERPLASIA, ENDOMETRIOID INTRAEPITHELIAL NEOPLASIA, AND ENDOMETRIOID CARCINOMA

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ABSTRACT

A cross-sectional study of 62 endometrial specimens consisting of 20 non-atypical hyperplasia cases, 20 endometrioid intraepithelial neoplasia cases, and 22 endometrioid carcinoma cases was conducted to determine the differences of mean AgNOR counts among these three groups. Mean AgNOR counts of each case was calculated by counting all black dots in the nuclei of 100 glandular cells and then the total number was divided by 100. mAgNOR counts were significantly higher in endometrioid carcinoma than the other two groups. Analysis of mAgNOR counts between the other two groups also showed that EIN was significantly higher than non-atypical hyperplasia.

INTRODUCTION

Endometrial carcinoma accounts for 6% of all malignant cases in female and is the most common genital malignancy. Most cases occur in postmenopausal age, although the increase of incidence in premenopausal age has been reported.[1,2] Endometrioid type carcinoma is the most common histologic type and sums up for three quarter of endometrial carcinoma cases.[3] Prior to endometrioid carcinoma, biopsy of endometrial lesions could reveal benign endometrial lesion, non-atypical hyperplasia, or atypical hyperplasia known as endometrioid intraepithelial neoplasia (EIN). Endometrial hyperplasia, the result from abnormal proliferation of endometrial glandular cells, is associated with high risk for cancer in long-term persistent case.[4,5] EIN, in particular tends to progress to endometrioid carcinoma. 22% of EIN cases progress to endometriod carcinoma later, whereas only 1,6% of non-atypical hyperplasia cases progress. While the features of EIN resemble those malignant cells in endometrioid carcinoma, EIN does not show invasion to the stroma. This slight difference sometimes gives confusion for pathologists in diagnosing endometrial hyperplasia and early endometrioid carcinoma.[6-8]
NOR (nucleolar organizer region) is DNA loop that projects into nucleoli in interphase cycle and encodes ribosomal RNA (rRNA). These rRNA is responsible for synthesizing cellular proteins that are stained selectively by silver colloid known as AgNOR. AgNOR staining is visualized as black dots under the light microscope. Increase of AgNOR counts is said to be associated with the advance of lesion aggressiveness.[9-11]

AgNOR technique is considered to be well known among pathologists in diagnostic studies. Previous studies showed increasing of mean AgNOR (mAgNOR) counts constantly from normal endometrium, endometrial hyperplasia to endometrioid carcinoma.10 In this study, mAgNOR counts were measured in non-atypical hyperplasia, EIN, and endometrioid carcinoma cases to find out the different value of each group.

METHODS

Sample Selection

This cross-sectional study was conducted on 62 endometrial cases in Anatomical Pathology Department of Universitas Sumatera Utara, Medan, Indonesia between 2015 and 2017. Inclusion criteria were paraffin-embedded blocks from post-curettage and post-hysterectomy specimens that were diagnosed as non-atypical hyperplasia, EIN, or endometrioid carcinoma histologically on hematoxylin-eosin stained section, and adequately representative specimens. Exclusion criteria were cluster of endometrial gland less than 10% of specimen area, and diameter of the cluster less than 2.1mm.5,12 Each case was reviewed by 3 observers. This study protocol was approved by the research ethics committee of Universitas Sumatera Utara.

AgNOR procedure

AgNOR staining was started by making a five-microns-thick section and deparaffinized it three times in xylene for three minutes each time. Then hydration process through decreasing concentrations of ethanol for three times in four minutes. Section then was installed in sodium citrate buffer and incubated for 20 minutes in autoclave at 120oC. Later the section was put at room temperature. At the other hand, solution of 2% gelatin in 1% formic acid was reacted with 25% silver nitrate and after that it was poured over the section at 37°C for 11 minutes. Reaction of the staining was stopped by washing the slide with deionized water. Dehydration process took place through increasing concentration of ethanol in short time. The section was cleared in xylene and mounted. Finally, Counting the number of blackdots of each glandular nucleus using the highest magnification (x1000) of light microscope needed immersion oil on top of the section. Total amount of blackdots from 100 glandular nuclei would be divided by 100 to earn the mean AgNOR value.

Statistical analysis

Analysis was done using the SPSS 22 version (SPSS Inc, Chicago) with 95% confidence interval. Analysis using Kruskal-Wallis test, and Mann-Whitney test with significant p<0.05 was performed.

RESULTS

In these 3 groups, age of each group was evaluated. There was significant difference in age among these 3 groups (p=0.001). Significant differences were found between endometrioid carcinoma and EIN (p=0.001), also between endometrioid carcinoma and non-atypical hyperplasia (p=0.004), whereas, there was no significant difference between age group of EIN and non-atypical hyperplasia (p=0.654). Age of endometrioid carcinoma group was found higher than the other two groups (Table 1).

Table 1. Age in Endometrial Lesion Groups

Variable	Non-atypical hyperplasia n=20	EIN n=20	Endometrioid carcinoma n=22	p
Age, years, median (minimum – maximum)	46 (29 – 76)	41 (27 – 51)	52 (32 – 85)	0.001*

*p<0.05

Out of 62 endometrial specimens, there were 20 (32%) cases of non-atypical hyperplasia, and 20 (32%) cases of EIN. The remaining 22 (36%) cases were endometrioid carcinoma. Twenty cases of non-atypical hyperplasia showed a range of 2.98-4.92 mAgNOR counts. The median of mAgNOR count in non-atypical

hyperplasia group was 3.54. mAgNOR counts of EIN group were in range of 3.70-6.01 with median mAgNOR count being 4.50. Cases of endometrioid carcinoma showed 6.58 median of mAgNOR count with range of 5.75-7.98 (Figure 1).

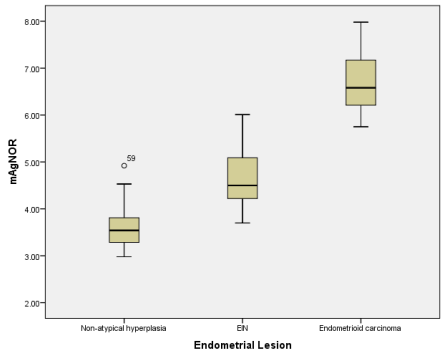

Figure 1. Distribution of mAgNOR Counts in Each Group of Endometrial Lesion

There were significant differences of mAgNOR counts among these 3 groups (p<0.001). Signifiantly higher mAgNOR counts in endometrioid carcinoma were found when compared to EIN (p<0.001) and non-atypical hyperplasia (p<0.001). Between EIN and non-atypical hyperplasia, there was also significant difference (p<0.001) of mAgNOR counts (Table 2).

Table 2. The Differences of mAgNOR Counts in Endometrial Lesion Groups

Variable	Non-atypical hyperplasia n=20	EIN n=20	Endometrioid carcinoma n=22	p
mAgNOR counts (minimum – maximum)	3,54 (2,98 – 4,92)	4,50 (3,70 – 6,01)	6,58 (5,75 – 7,98)	<0.001*

*p<0.05,

DISCUSSION

The incidence of endometrial hyperplasia and endometrioid carcinoma is more common in perimenopausal and postmenopausal women.[13,14] Perimenopausal signs are very common in their forties although they can occur in their late thirties or fifties.[15] In the perimenopausal period, intermittent ovulatory or even chronic anovulation occurs, therefore low progesterone levels are due to the absence of the corpus luteum. The ovary still produces estrogen which causes the endometrial proliferation to continue so endometrial thickening exceeds its blood supply resulting in focal necrosis and shedding. Uneven shedding and bleeding tend to be irregular, prolonged and severe. Chronic endogenous estrogen stimulation in the endometrium is not followed by adequate progesterone levels may cause endometrial hyperplasia and endometrioid carcinoma.16 In this study, the median age of endometrioid carcinoma is 52 years with the youngest age of 32 years. Whereas, non-atypical hyperplasia and EIN appeared on younger age than endometrioid carcinoma group. Endometrioid carcinoma mainly affects postmenopausal women. This carcinoma is not common in women under the age of 45 years but also not rare, accounting for about 5% of all cases.[17,18]

This study aims to determine the differences of mAgNOR counts in non-atypical hyperplasia, EIN, and endometrioid carcinoma cases. Increasing of AgNOR counts was said to be correlated with the increase of cellular proliferation where protein synthesis was needed. Previous studies revealed that mAgNOR counts of endometrioid carcinoma were higher than both groups of endometrial hyperplasia, and mAgNOR counts of EIN were also higher than non-atypical hyperplasia. This study was in line with previous studies in which this study showed that endometrioid carcinoma had the highest mAgNOR counts compared to all endometrial hyperplasia.10,11 Between endometrial hyperplasia lesions, the mAgNOR counts of EIN was higher than the mAgNOR counts of non-atypical hyperplasia. The mAgNOR counts in this study appeared to have an increasing trend along with further neoplastic changes.

CONCLUSION

The mAgNOR difference was seen among the three groups of endometrial lesions with an increased mAgNOR counts corresponding to the aggressiveness of the three lesions.

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