Original Article

Expression of AgNOR in Benign, Borderline and Malignant Epithelial Tumors of Ovary and Its Correlation with Tumor Grading

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Abstract

***Background**: Surface epithelial tumor is the most common type of ovarian tumor making up 65% of all ovarian neoplasms. The proliferative activity of tumor can be estimated by many methods and the analysis of silver binding nucleolar organizer region (AgNOR) is one of them. Two types of AgNOR counts were calculated – mAgNOR and pAgNOR.* ***Objective**: To find out expression of AgNOR staining in benign, borderline and malignant epithelial tumors of ovary and its correlation with tumor grading.* ***Materials and Methods**: This cross-sectional study was conducted in the department of Pathology, Sir Salimullah Medical College, Dhaka from March 2018 to July 2020. In this study 70 diagnosed cases of benign, borderline and malignant epithelial tumors of ovary were enrolled. Corresponding paraffin blocks were collected. Ethical practice was ensured in every step of the study. Statistical analysis was carried out as required. Statistical significance was considered at <0.05.* ***Results**: Most of the patients were in 31−40 years age group. Most commonly observed epithelial tumors were benign in nature followed by malignant tumors and only two cases were borderline. The mean mAgNOR in malignant tumors was 3.62±0.57, with benign tumors was 1.68±0.53 and with borderline tumors was 1.65±0.21. Progressive increase in AgNOR expression was noted from benign to malignant epithelial ovarian tumors. The mean mAgNOR found in Grade I, Grade II and Grade III were 3.29±0.37, 3.76±0.59 and 4.33±0.15 respectively. Significant differences were found among the all groups and when compared between Grade I and Grade II, also between Grade I and Grade III. The mean pAgNOR found in Grade I, Grade II and Grade III were 13.54±3.05, 16.19±4.35 and 21.33±1.15 respectively. Significant differences were found among the groups and also when compared between Grade I and Grade III. There was significant positive correlation between histological grading of ovarian tumor with mAgNOR (r=0.589; p=0.001) and with pAgNOR (r=0.510; p=0.003).* ***Conclusion**: mAgNOR and pAgNOR expressions were significantly elevated in malignant tumors compared to benign and borderline tumors. mAgNOR and pAgNOR are useful markers of cellular kinetics and good prognostic factors in epithelial tumors of ovary.*

***Key words**: Malignant; Benign; Borderline; Ovarian tumor; mAgNOR; pAgNOR*

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Introduction

Ovarian cancer is the leading cause of gynecological cancer death in women and impacts female life and health all over the world.[1] It is the fourth cause of all deaths due to malignant neoplasm in women after cancer of the breast, lung and bowel. Its common histological type is serous cystadenocarcinoma.[2]

Neoplastic tumors arising from the surface epithelium of the ovary comprise a broad spectrum of neoplasms ranging from serous to endometroid, mucinous, transitional, clear cell and undifferentiated tumor types.[3] Surface epithelial tumor is the most common type of ovarian tumor making up 65% of all ovarian neoplasms (WHO classification of ovarian neoplasm 2018).

The proliferative activity of neoplasms can be estimated by many methods and the analysis of silver of [4] nucleolar organizer regions (NOR) are segments of DNA that transcribe to ribosomal RNA and are situated on short arms of the acrocentric chromosomes 13, 14, 15, 21 and 22. The number of NOR reflects the transcriptional activity of cells and is related to cell cycle stage.[5,6] After sliver staining NORs can be easily identified as black dots exclusively situated throughout nuclear area and are called AgNOR.[7] The amount of AgNOR is proportional to the proliferative activity of neoplastic cells. It is related to the cell cycle progressively increasing from G0 to S phase.[8] In general, highly malignant tumors present greater amount of AgNORs than benign or less malignant tumors.[9,10]

Although there are a few studies regarding ovarian cancer in our country but to the best of our knowledge there are little available data regarding the role of AgNOR in the grading of ovarian tumor. Therefore, the present study is aimed to expression of AgNOR in benign, borderline and malignant epithelial tumors of ovary and its correlation with tumor grading.

Materials and Methods

This cross-sectional study was carried out on 70 patients with epithelial tumors of ovary from Pathology department of Sir Salimullah Medical College & Mitford Hospital, Dhaka during March 2018 and July 2020. Patients with histopathologically diagnosed cases of benign, borderline and malignant epithelial tumors of ovary were collected in this study. Patients declining consent, patients who were treated with radiotherapy or chemotherapy were excluded from the study. Ethical clearance was obtained from the Research Committee of Sir Salimullah Medical College (SSMC). Objective of the study along with its procedure, alternative diagnostic methods, risk and benefits were explained to the patients in easily understandable local language and then informed consent was taken from each patient. It was assured that all records would be kept confidential.

The resected specimens were collected in 10% buffered formalin. After overnight fixation tissue blocks were made following paraffin embedded technique. For each case one section from specimen blocks was stained with H&E for histopathological diagnosis. Another 4 micrometer thick section was dewaxed and processed for AgNOR staining. The diagnosis was done by histopathological examination with haematoxylin and eosin stain and categorized according to WHO classification. WHO grading was done.

AgNOR count: Two types of AgNOR count were calculated - mAgNOR and pAgNOR on each AgNOR stained slides.

mAgNOR: mAgNOR count is the mean number of AgNORs dot in 100 tumor nuclei. AgNOR dots are counted on hundred randomly selected cell nucleus.

pAgNOR: This is the percentage of nuclei exhibiting five or more AgNORs/nucleus/100 cells called proliferative index (pAgNOR).

Statistical analysis was carried out by using the Statistical Package for Social Sciences Version 25.0 for windows (SPSS INC Chicago, Illinois, USA). A descriptive analysis was performed for all data. The mean values were calculated for continuous variables. The quantitative observations were indicated by frequencies and percentages. Chi square test, one way ANOVA followed by Bonferroni and Spearman's correlation co-efficient test were performed. p value <0.05 was considered as statistically significant.

Results

Among 70 study subjects, 36 (51.4%) had benign, 32 (45.7%) malignant and 2 (2.9%) had borderline ovarian tumors. The mean age of the study population was 38.04 years (SD ± 10.40) ranging from 19 to 70 years. Highest number of patients (41.4%) were in age group 31−40 years and lowest number of patients (5.7%) were younger than 20 years. Out of 70 cases, 32 cases (45.7%) of ovarian tumors were malignant histopathologically, 36 cases (51.4%) were benign and other two cases (2.9%) were borderline. Mean age of subjects of malignant, benign and borderline tumors was (44.31±9.54) years, (33.42±8.01) years and (39.00±1.41) years respectively (Table I). The age of patients with malignant tumors was higher than patients with benign and borderline tumors.

Table I: Distribution of types of tumors according to age (n=70)

Tumor types	Number (%)	Age (years) Mean±SD	p value
Benign	36 (51.4)	33.42±8.01	
Borderline	02 (2.9)	39.00±1.41	0.001[s]
Malignant	32 (45.7)	44.31±9.54	

s=significant, p value reached from ANOVA test

Among patients with malignant tumors, 27 cases (84.4%) were multiparous and 5 cases (15.6%) were nulliparous. Among patients with benign tumors, 13 (40.6%) were multiparous, 23 (71.9%) were nulliparous. Among patients with borderline tumors, one (50.0%) was multiparous and one (50.0%) was nulliparous (Table II). In the present study, higher incidence of malignant tumors was found in multiparous women. Among the study subjects with malignant tumors, 11 cases were of postmenopausal, one case had amenorrhea for one year, 12 cases were on regular menstrual cycle and 8 cases were irregular. In cases with benign tumors, one case was postmenopausal, 30 cases were on regular menstrual cycle and 5 cases were irregular. In cases with borderline tumors, 2 cases were on regular menstrual cycle. There was statistical significant difference among malignant, benign and borderline ovarian tumors regarding menstrual history. Among them 7 cases were malignant with positive family history. Twenty five cases were malignant, thirty six cases were benign and two cases were borderline with no family history.

Table II: Distribution of the study subjects according to parity, menstrual history and family history of tumors (n=70)

	Benign (n=36)	Borderline (n=2)	Malignant (n=32)	p values
	Number (%)	Number (%)	Number (%)	
Parity				
Multiparous	13 (40.6)	1 (50.0)	27 (84.4)	0.001[s]
Nulliparous	23 (71.9)	1 (50.0)	5 (15.6)	
Menstrual history				
Postmenopausal	1 (3.1)	0 (0.0)	11 (34.4)	0.004[s]
Regular	30 (93.8)	2 (100.0)	12 (37.5)	
Irregular	5 (15.6)	0 (0.0)	8 (25.0)	
Menorrhagia	0 (0.0)	0 (0.0)	1 (3.1)	
Family history of tumors				
Positive	0 (0.0)	0 (0.0)	7 (21.9)	0.010[s]
Negative	36 (100.0)	2 (100.0)	25 (78.1)	

s=significant, p value reached from Chi-square test

According to USG findings, among the complex masses, 53.1% cases were malignant. In case of cystic mass, 12.5% were malignant, 36.1% were benign and 50% were borderline. In mass lesion, only 34.4% were malignant, 58.3% were benign and 50.0% were borderline (Table III).

Table III: Distribution of the study patients according to USG findings (n=70)

USG findings	Benign (n=36) Number (%)	Borderline (n=2) Number (%)	Malignant (n=32) Number (%)	p value
Complex mass	2 (5.6)	0 (0.0)	17 (53.1)	
Mass lesion only	21 (58.3)	1 (50.0)	11 (34.4)	0.0001[s]
Cystic mass	13 (36.1)	1 (50.0)	4 (12.5)	

s=significant, p value reached from Chi-square test

Of the total 70 cases of ovarian tumors, 22 cases had malignant serous cystadenocarcinoma, benign brenner tumors in 4 cases, malignant mucinous cystadenocarcinoma in 8 cases, benign serous cystadenoma in 15 cases, benign mucinous cystadenoma in 17 cases, malignant endometrioid adenocarcinoma in 2 cases and borderline mucinous cystadenoma in 2 cases (Table IV).

Table IV: Distribution of the study subjects according to histopathological diagnosis (n=70)

Histopathological tumor types	Benign (n=36) Number (%)	Borderline (n=2) Number (%)	Malignant (n=32) Number (%)
Serous cystadenoma	15 (41.7)	0 (0.0)	0 (0.0)
Mucinous cystadenoma	17 (47.2)	0 (0.0)	0 (0.0)
Benign Brenner tumor	4 (11.1)	0 (0.0)	0 (0.0)
Borderline mucinous cystadenoma	0 (0.0)	2 (100.0)	0 (0.0)
Serous cystadenocarcinoma	0 (0.0)	0 (0.0)	22 (68.8)
Mucinous cystadenocarcinoma	0 (0.0)	0 (0.0)	8 (25.0)
Endometrioid adenocarcinoma	0 (0.0)	0 (0.0)	2 (6.3)

Mean mAgNOR malignant was 3.62±0.57, benign was 1.68±0.53 and borderline was 1.65±0.21 respectively and pAgNOR in malignant, benign and borderline cases were 15.59±4.24, 3.61±2.25 and 2.50±0.70. There was statistical significant difference between these two groups ($p<0.05$) (Table V).

Table V: Relation of mAgNOR and pAgNOR with tumor types (n= 70)

Tumor types	mAgNOR	pAgNOR
	Mean±SD	Mean±SD
Benign (A)	1.68±0.53	3.61±2.25
Borderline (B)	1.65±0.21	2.50±0.70
Malignant (C)	3.62±0.57	15.59±4.24
Statistical analysis	*p values*	*p values*
A vs B vs C	0.001[s]	0.001[s]
A vs B	0.937[ns]	0.496[ns]
A vs C	0.001[s]	0.001[s]
B vs C	0.001[s]	0.001[s]

s=significant, ns=not significant, p value reached from ANOVA test

To measure the mAgNOR in cases of various grade of ovarian tumors, one way ANOVA followed by Bonferroni and Spearman's correlation co-efficient test was performed. Mean mAgNOR in grade I, grade II and grade III were 3.29±0.37, 3.76±0.59 and 4.33±0.15 respectively. Significant differences were found among the groups and when compared between grade I and grade II, also between grade I and grade III (Table VI). To measure the pAgNOR in cases of various grades of ovarian tumors, one way ANOVA followed by Bonferroni and Spearman's correlation co-efficient test was performed. Mean pAgNOR found in grade I, grade II and grade III were 13.54±3.05, 16.19±4.35 and 21.33±1.15 respectively. Significant differences were found among the groups and also when compared between grade I and grade III.

Table VI: Relation of mAgNOR and pAgNOR with grade (n=32)

Grading	mAgNOR	pAgNOR
	Mean±SD	Mean±SD
Grade I (A)	3.29±0.37	13.54±3.05
Grade II (B)	3.76±0.59	16.19±4.35
Grade III (C)	4.33±0.15	21.33±1.15
Statistical analysis	p values	p values
A vs B vs C	0.004[s]	0.008[s]
A vs B	0.019[s]	0.075[ns]
A vs C	0.004[s]	0.008[s]
B vs C	0.122[ns]	0.063[ns]

s=significant, ns=not significant, p value reached from ANOVA test

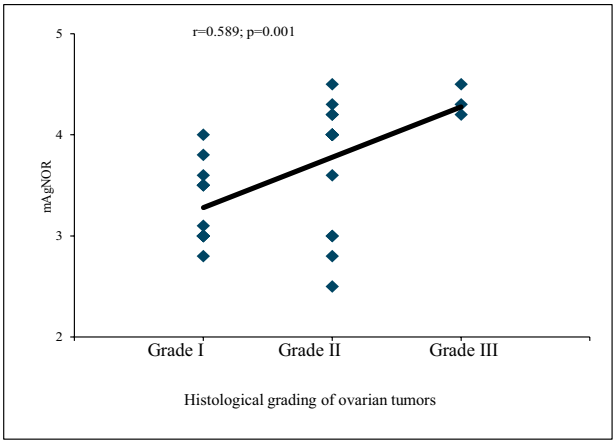

Fig 1. Correlation of histological grading of ovarian tumors with mAgNOR. Here a positive significant relation is achieved (r=0.510; p=0.003)

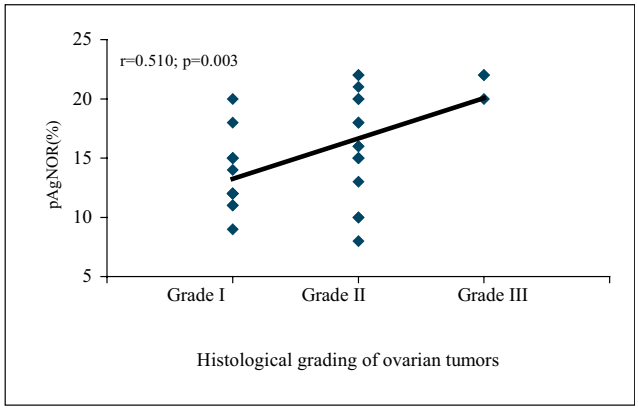

Fig 2. Correlation of histological grading of ovarian tumors with pAgNOR. Here a positive significant relation is achieved (r=0.510; p=0.003)

Discussion

Ovarian cancer (OC) is the seventh most commonly diagnosed cancer among women in the world and the tenth most common in China. Epithelial OC is the most predominant pathologic subtype, with five major histotypes that differ in origination, pathogenesis, molecular alterations, risk factors and prognosis. Several reproductive and hormonal factors such as parity, oral contraceptive use and lactation may lower risk, while others such as older age at menopause and hormone replacement therapy confer increased risks.[11]

In this study, it was observed that the mean age of the study population was 38.04±10.40 years with age of patients varying from 19 to 70 years and the highest number of patients were in age group 31−40 years and the lowest number of patients were in group younger than 20 years. Dhar et al[12] found mean age 38.2±7.31 years with a range of 16 to 69 years and most patients were more than 30 years old, which is almost similar to the present study.

Regarding histopathological diagnosis of ovarian tumor, we found that 51.4% of ovarian tumors were benign, 2.9% were borderline and 45.7% were malignant. Mean age of benign, borderline and malignant tumors was 33.42±8.01 years, 39.00±1.41 years and 44.31±9.54 years respectively. The age of patients with malignant tumor was higher than patients with borderline and benign tumor. Similarly, Okugawa et al[13] found in their study that 51.0% cases have benign tumors, 4.0% have tumors of low

malignant potential but 16.0% cases have malignant tumors and 28.0% cases have tumor-like lesions.

It was observed that among patients with benign tumors, 13 (40.6%) were multiparous, 23 (71.9%) were nulliparous. Among patients with borderline tumor, 1 (50.0%) were multiparous and 1 (50.0%) were nulliparous. Among patients with malignant tumor, 27 cases (84.4%) were multiparous and 5 cases (15.6%) were nulliparous. Higher incidence of malignant tumors was found in multiparous women in the present study. Dhar et al[12] found 12.0% nulliparous and 80.0% were multiparous which is comparable with the present study.

Regarding the menstrual history of ovarian tumor cases, postmenopausal and history of regular menstruation were significantly higher in malignant ovarian tumor cases. Cirillo et al[14] found that irregular cycle was a marker for higher risk of ovarian cancer and women with a history of menstrual irregularities were twice as likely to develop ovarian cancer. In another study, Shen et al[15] observed a total of 854 patients with epithelial ovarian cancers, out of which 42.0% patients were diagnosed before menopause and 58.0% patients were diagnosed after menopause.

Of the total tumors, positive family history of ovarian tumors was significantly ($p<0.05$) associated with malignant ovarian tumors in this study. Ovarian cancer run in families. About 5.0% to 10.0% of ovarian cancers are a part of family cancer syndromes (American Cancer Society, cancer.org 1.800.227.2345), which support findings of the present study.[16]

According to USG findings, complex mass was significantly ($p<0.05$) more common in malignant ovarian tumors in this study. A study of Cohen[17] found that most of the malignancy occurred in complex mass, which is consistent with the present study.
According to Kumar et al[18] serous neoplasm accounted for approximately 40.0%, mucinous 20−25%, endometrioid 10.0% of all ovarian neoplasms. In this study serous cyst adenocarcinoma was 68.8% and mucinous cyst adenocarcinoma was 25.0%.

In this study, it was observed that the mean mAgNOR and pAgNOR were significantly ($p<0.05$) higher in malignant compared to benign groups and also compared to borderline groups. In epithelial ovarian tumors mAgNOR and pAgNOR increase from benign to borderline and malignant neoplasm.[19,20]

In this study it was observed that the mean mAgNOR was significantly ($p<0.05$) higher in grade III compared to grade I and also compared to grade II (Fig 1). However, the mean pAgNOR was significanty different among the grades and significantly ($p<0.05$) higher in grade III with compared to grade I.

Gottwald et al[21] found that mAgNOR and pAgNOR were positively correlated with tumor grading. An increased mAgNOR value positively correlates with the number of acrocentric chromosomes, increased amount of DNA and aneuploidy.[22] In this present study, a significant positive correlation ($r=0.589$; $p=0.001$) was achieved between histological grading of ovarian tumor and mAgNOR. There was also a positive significant correlation obtained ($r=0.510$; $p=0.003$) with pAgNOR.

This study was undertaken to find out expression of AgNOR staining in benign, borderline and malignant epithelial tumor of ovary. mAgNOR and pAgNOR expressions were significantly elevated in malignant tumors compared to benign and borderline tumors and these are useful markers for both cell proliferation and malignancy. Hence the AgNOR count can be used as supplementary test for the difficult histoprognostic evaluation in different types of malignancy. Further studies on larger numbers of samples are required to confirm the association of AgNOR with malignancy. Thus the AgNOR numbers are of clinical importance in malignancies of ovary and their estimation should be regarded as a valuable adjunct, in addition to the histopathological criteria for evaluation of proliferative activity.

The study population was selected from a selected hospital in Dhaka city, so that the results of the study may not reflect the exact picture of the country. Small number of borderline tumors were also a limitation of the present study.

AgNOR staining may be done in ovarian cancer patients to better characterize them. Aggressive

tumors often have higher mAgNOR and pAgNOR values. Further studies can be undertaken including large number of borderline ovarian tumors.

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