Original Article

Expression of AgNOR in Benign, Borderline and Malignant Epithelial Tumors of Ovary and its Correlation with Serum CA 125

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Abstract

Background*: CA 125, as a biomarker, holds promise in a wide range of applications, including risk assessment, early detection, diagnosis, prognosis, monitoring, and therapy.* ***Objective****: To find out expression of AgNOR staining in benign, borderline and malignant epithelial tumors of ovary and its correlation with serum CA 125.* ***Materials and Methods****: The cross-sectional analytical study, spanning from March 2018 to July 2020 in the Department of Pathology, Sir Salimullah Medical College, Dhaka included 70 diagnosed cases of ovarian epithelial tumors with known CA 125 status. Paraffin blocks and CA 125 reports were gathered, ensuring ethical practices throughout.* ***Results****: The mean CA 125 levels in patients with benign was 61.70±30.75 U/mL, in borderline group 65.0±7.07 U/mL and in malignant cases 463.67±249.48 U/mL. CA 125 level was significantly higher in malignant tumors compared to benign and borderline tumors (p<0.001). There was significant positive correlation between CA 125 with mAgNOR (r=0.821; p=0.001) and with pAgNOR (r=0.853; p=0.001).* ***Conclusion****: Elevated CA 125 levels were notably significant in malignant tumors compared to benign and borderline cases. A strong, positive correlation existed between CA 125 and both mAgNOR and pAgNOR.*

Key words*: Benign, Borderline, mAgNOR, Malignant, Ovarian tumor, pAgNOR, Serum CA 125*

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Introduction

Epithelial ovarian cancer, the most prevalent type within its category, originates in the epithelial tissue enveloping the ovary. Alternatively, it may emerge in the lining of the fallopian tube or the peritoneum, covering abdominal organs. With over 30 distinct types of ovarian cancer, there exists a considerable variation in their incidence and prognosis. The outlook for women diagnosed with different forms of ovarian cancers varies widely. Notably, studies classify fallopian tube and primary peritoneal cancers as subtypes falling under the broader category of epithelial ovarian cancer.[1]

Ovarian cancer incidence varies across age and race groups, with the highest rates observed in Eastern Europe−11.4 per 100,000, and 6.0 per 100,000 in Central Europe. China has a relatively low incidence rate−4.1 per 100,000. Transitioned countries exhibit higher ovarian cancer incidence, with approximately 30.0% of cases occurring in European countries.[2]

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Notably, Singapore, Kazakhstan, and Brunei have the highest standardized incidence rates among Asian countries.[3]

A major risk factor for epithelial ovarian cancer (EOC) is a family history of breast and/or ovarian cancer (HBOC), with both EOC and primary peritoneal cancer (PPC) traditionally treated similarly despite noted molecular and clinical differences.[4] First-degree relatives of individuals with ovarian cancer face a three-fold increased risk, particularly if diagnosed under 50 years, and serous ovarian cancer poses a higher relative risk for family members.[5] Hormonal and reproductive factors also play a significant role; a higher lifetime number of menstrual cycles increases EOC risk, while factors reducing ovulation, such as pregnancy and oral contraceptives, are protective.[6] Hormone replacement therapy, increased height, weight, and body mass index, but not diet or alcohol, carry modest risks.[7,8] Smoking associates with mucinous ovarian cancer, and endometriosis elevates the risk of clear cell and endometrioid ovarian cancer.[9,10]

In 2020, ovarian cancer (OC) was 313,959 new cases and 207,252 deaths globally. Asia reported the highest OC mortality at 54.4%, while Europe followed with 21.3%. In Brunei Darussalam, OC ranked as the fifth most prevalent cancer among females.[11] Similar to many cancers, OC incidence exhibits global variation. According to GLOBOCAN 2020 estimates, age-standardized (world) incidence and mortality rates for OC in Brunei Darussalam were 17.4 per 100,000 and 7.4 per 100,000 respectively.[12] These figures highlight the worldwide impact of OC, emphasizing regional differences in both occurrence and fatality rates.

AgNORs have proven to be valuable proliferation markers across various cancers, including ovarian, endometrial, cervical, breast, gastric, colorectal, oral, bladder, prostate, hepatocellular, and salivary gland cancer.[13,14] These studies suggest that incorporating various markers of morphometric nuclear characteristics and AgNOR values could enhance the differential cytodiagnosis of benign, borderline, and malignant serous ovarian tumors.

Serum CA 125, also known as cancer antigen 125, carcinoma antigen, or carbohydrate antigen 125, is a protein encoded by the MUC16 gene often referred to as MUCIN16.[15,16] Widely recognized as a primary biomarker for ovarian cancer detection, CA 125 proves particularly valuable in clinical assessments.[17] Approximately 90% of women with advanced ovarian cancer exhibit elevated CA 125 levels in their blood serum, establishing it as a reliable indicator of post-symptom onset.[18] CA 125, a glycoprotein synthesized by neoplastic cells with epithelial differentiation, plays a crucial role in distinguishing between benign and malignant tumor processes. Higher pre-operative serum CA 125 levels are significantly associated with malignant tumors[19] and its levels correlate with histological grade and disease stage, providing valuable insights into the biological potential of ovarian lesions.[20]

The purpose of this study is to find out the expression of AgNOR in benign, borderline and malignant epithelial tumors of ovary and its correlation with serum CA 125.

Materials and Methods

This prospective cross-sectional analytical study enrolled 70 patients with ovarian epithelial tumors at Sir Salimullah Medical College & Mitford Hospital, Dhaka between March 2018 and July 2020. The study included histopathologically diagnosed cases of benign, borderline, and malignant epithelial ovarian tumors. The study's objective, procedures, alternative diagnostic methods, risks, and benefits were explained in the local language to patients. Informed consent was obtained with assurance of confidentiality for all records. Patients declining consent or undergoing radiotherapy/chemotherapy were excluded. Ethical clearance was obtained from the Institutional Ethical Review Board of Sir Salimullah Medical College.

Study procedure: Resected specimens were

preserved in 10% buffered formalin. Following overnight fixation, tissue blocks were created using the paraffin-embedding technique. A histopathological diagnosis was performed on one section stained with H&E. Another 4-micrometer-thick section was dewaxed and subjected to AgNOR staining. Histopathological examination, utilizing hematoxylin and eosin stain, categorized diagnoses following the WHO classification of ovarian neoplasm.

mAgNOR: The mAgNOR count represents the average number of AgNOR dots within 100 tumor nuclei. The counting of AgNOR dots is conducted on a randomly chosen set of one hundred cell nuclei.

pAgNOR: The proliferative index (pAgNOR) is defined as the percentage of nuclei that display five or more AgNORs per nucleus per 100 cells.

CA 125: Normal value range from 0-35 U/mL[18].

Statistical analysis: The statistical analysis utilized the Statistical Package for Social Sciences Version 26.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Mean values were computed for continuous variables, and statistical tests including ANOVA and Spearman's correlation coefficient were conducted. A significance level of $p < 0.05$ was adopted to determine statistical significance.

Results

The age of the studied population varied from 19 to 70 years and most of the patients' age belonged to 31-40 years.

CA 125 levels in ovarian tumors

Out of 70 cases of benign, borderline and malignant ovarian tumors, mean CA 125 levels in patients with benign, borderline and malignant tumor were 61.70±30.75 U/mL, 65.0±7.07 U/mL and 463.67±249.48 U/mL respectively. CA 125 level was significantly higher in malignant tumors compared to benign and borderline ($p<0.001$) (Table I). Fig 1 shows correlation between CA 125 and mAgNOR. Here a positive significant relation is achieved ($r=0.821$; $p=0.001$) and Fig 2 shows correlation between CA 125 and pAgNOR. Here also a positive significant relation is achieved ($r=0.853$; $p=0.001$)

Table I: Relation of CA 125 with tumor type (n= 70)

Tumor type	CA 125 (U/mL)	
	Mean±SD	Range (min-max)
Benign	61.70±30.75	32.0–200.0
Borderline	65.0±7.07	60.0–70.0
Malignant	463.67±249.48	185.0–1201.0
p value	0.001	

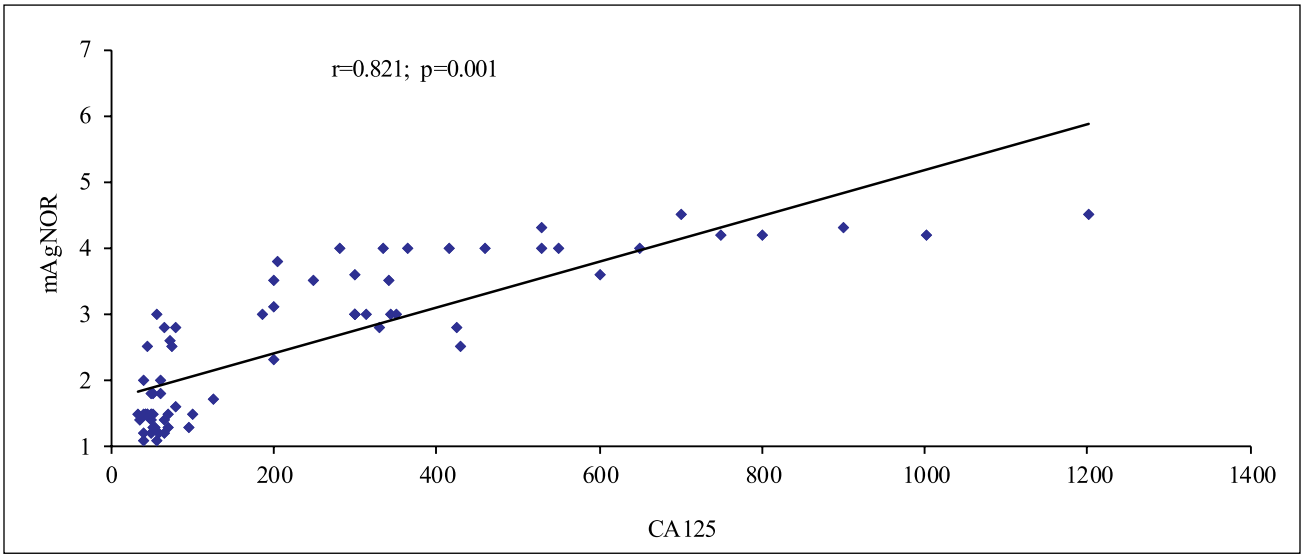

Fig 1. Correlation between CA 125 and mAgNOR

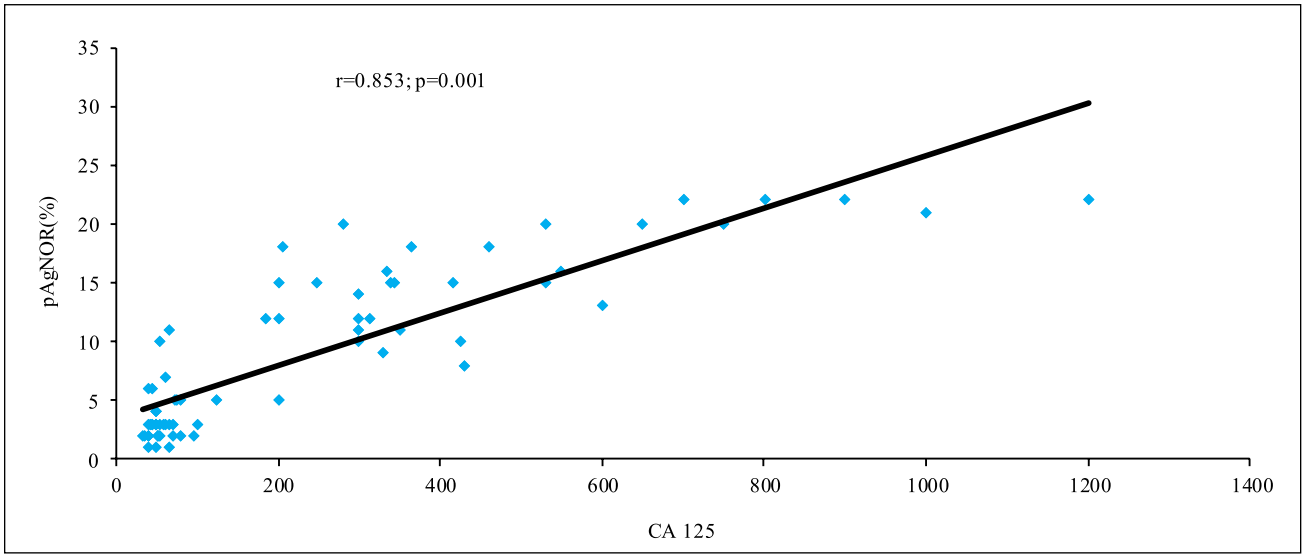

Fig 2. Correlation between CA 125 and pAgNOR

Discussion

Globally, ovarian cancer ranks as the eighth most prevalent cancer among women and the 18th most common cancer overall. In 2020, there were over 313,000 new cases and more than 207,000 deaths attributed to ovarian cancer.[2,21] Epithelial ovarian cancer (OC) is the primary pathological subtype, comprising five major histotypes with distinct origins, pathogenesis, molecular changes, risk factors, and prognosis. Rare inherited mutations contribute to genetic susceptibility while genome-wide association studies reveal 29 common susceptibility alleles, including 14 subtype-specific ones. Factors like parity, oral contraceptive use, and lactation may reduce OC risk while older age at menopause and hormone replacement therapy elevate risks. The relationship between the expression of Silver-staining Nucleolar Organizer Regions (AgNOR) in various types of ovarian tumors and the levels of serum CA 125, a biomarker for ovarian cancer. The research aims to understand the role of AgNOR in the progression of ovarian tumors from benign to malignant stages. It investigates whether there is any correlation between AgNOR expression and CA 125 levels, which could potentially enhance the diagnostic accuracy for ovarian cancer. The findings could contribute to improved early detection and treatment strategies for ovarian cancer. This study underscores the importance of integrated diagnostic approaches in oncology.

In this investigation 70 cases of ovarian tumors were analyzed, which included benign, borderline, and malignant types. The average CA 125 level was found to be 61.70±30.75 U/mL in patients with benign tumors, 65.0±7.07 U/mL in those with borderline tumors, and significantly higher at 463.67±249.48 U/mL in patients with malignant tumors. Malignant tumors had a significantly higher CA 125 level than benign and borderline tumors ($p<0.001$). Chen et al[22] also found that the malignant group had a much higher serum CA 125 level than the benign group ($p<0.05$), with a mean of 184.62 U/mL and 52.31 U/mL respectively. CA 125 can distinguish between benign and malignant tumor processes. Moreover, the serum CA 125 levels before surgery were significantly higher in serous tumors and advanced lesions.[19]

A study conducted in the UK found that women with normal CA 125 levels more frequently had indolent tumors and were more commonly diagnosed at an early stage in the course of the disease.[23] Another study showed that CA 125 is a useful test for ovarian cancer detection in primary care, particularly in women aged 50 years and older.[24] However, some studies have also found that CA 125 levels can be elevated in non-malignant conditions, such as endometriosis, cysts, and inflammation, and that AgNOR counts can vary depending on the histological type and grade of the

tumor.[25] Therefore, the diagnostic and prognostic value of these markers may be limited by their specificity and sensitivity, and they should be interpreted in conjunction with other clinical and radiological features. The disparity between our findings and these international studies could be attributed to several factors. These include differences in the patient population, variations in the methodology used for CA 125 level measurement, and the stage of the disease at the time of diagnosis. Further research is needed to explore these differences and their implications for the diagnosis and treatment of ovarian tumors.

The present study has revealed significant positive correlations between CA 125 and both mAgNor and pAgNor. Specifically, the Pearson's correlation coefficients were found to be 0.853 (p=0.001) and 0.821 (p=0.001) respectively. These findings suggest a strong association between the expression of AgNOR and the levels of serum CA 125 in ovarian tumors, regardless of whether they are benign, borderline, or malignant. Examining epidemiological literature reveals CA 125 as the premier tumor marker in ovarian cancer, crucial for tracking responses to chemotherapy, relapses, and disease progression.[26] Additional research indicates that women with normal CA 125 levels often present with indolent tumors and are more frequently diagnosed at an early stage in the disease's progression.[23] However, it is important to note that while these studies underscore the[23] significance of CA 125 in ovarian cancer prognosis and diagnosis, they do not specifically investigate its correlation with AgNOR expression. Therefore, the findings of the present study add a new dimension to our understanding of ovarian tumors, potentially paving the way for improved diagnostic and prognostic strategies.

This study aimed to assess AgNOR staining expression in ovarian epithelial tumors of varying malignancy (benign, borderline, and malignant) and its correlation with preoperative serum CA 125 levels. Preoperative CA 125 was notably higher in malignant tumors than in benign and borderline tumors. Positive correlations were observed between CA 125 and both mAgNor and pAgNor. Larger sample sizes are warranted for confirmation of the AgNOR-malignancy association.

To enhance the characterization of ovarian cancer patients, AgNOR staining can be employed. Aggressive tumors typically exhibit elevated mAgNOR and pAgNOR values. Further research endeavors could expand upon these findings, involving a larger cohort of borderline ovarian tumors.

Limitations

Limitations of the study encompass the confined study population drawn exclusively from selected Dhaka city hospitals, possibly lacking representation for the entire country, and a limited number of borderline tumors. Subsequent research efforts should aim for a broader investigation into borderline tumors to enhance the generalizability and depth of findings.

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