Evaluation of CD56 Expression in Epithelial Ovarian Carcinoma and its Correlation with Histopathological Grading and Staging

*Zhumur M,[1] Rahman A,[2] Rahman MM,[3] Jinnah MA,[4] Jahra FT,[5] Dewan MR,[6] Jeba R[7]

Abstract

Background: Epithelial ovarian carcinoma (EOC) is the major subtype of ovarian cancer which leads to most of the gynecological malignancy related death in women. As majority of the patients are diagnosed at advanced stages, the overall survival is poor. Despite improvements in surgery & chemotherapy, treatment failure and unfavorable clinical outcome commonly occur. The reason behind this is frequent chemotherapeutic resistance. Recently, CD56 (neural cell adhesion molecule) has been proposed as a potential target for antibody-based therapy. CD56 is a cell surface glycoprotein which is widely expressed in different carcinoma including EOC. Several studies showed that CD56 is significantly associated with high grade and advanced tumor stage in EOC and is a potential prognostic marker for poor overall survival.

Objectives: The aim of this study was to evaluate CD56 expression in EOC and its correlation with histopathological grading and staging.

Methods: This cross-sectional study was conducted in the Department of Pathology, Dhaka Medical College, Dhaka from March 2018 to February 2020. In this study, fifty biopsy samples of EOC were collected, processed, stained with haematoxyline and eosin. Immunostaining for CD56 was done in formalin fixed paraffin embedded tissue.

Results: Most commonly observed epithelial ovarian carcinoma was serouscarcinoma (68%). Most of the tumors were in grade two (44%) and stage three (44%). Seventeen (34%) cases stained positive for CD56 amongst which most of the cases (22%) were moderately positive. The study revealed that CD56 expression positively correlated with histopathological grading (p=0.041) and staging(p=0.015).

Conclusion: CD56 expression positively correlates with histopathological grading and staging in epithelial ovarian carcinoma.

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Keywords: CD56, NCAM, Epithelial ovarian carcinoma, Ovarian cancer.

1. **Dr. Mahbuba Zhumur, Lecturer, Department of Pathology, Dhaka Medical College. m.zhumur@gmail.com.*
2. *Dr. Arebia Rahman, Specialist - Pathology, Square Hospital Ltd.*
3. *Dr. Md. Mizanur Rahman, Lecturer, Department of Pathology, Dhaka Medical College.*
4. *Dr. Mohammed Shahed Ali Jinnah, Associate Professor and Head, Department of Pathology, Dhaka Medical College.*
5. *Dr. Fatima Tuj Jahra, Assistant Professor, Department of Pathology, Aichi Medical College.*
6. *Prof. Dr. Md. Rezaul Karim Dewan, Professor and Head, Department of Pathology, Greenlife Medical College.*
7. *Dr. Ruksana Jeba, Associate Professor, Department of Pathology, Dhaka Medical College.*

**For correspondence*

Introduction

Ovarian cancer is the deadliest of all cancer in female genital tract. Globally, it is the seventh most common cancer in women and eighth most common cause of their cancer mortality.[1] In Bangladesh ovarian cancer is the 12th most common cancer which accounted for 2% of cancer related death in 2018.[2] Approximately ninety percent of all ovarian carcinoma are epithelial ovarian carcinoma (EOC) which is usually diagnosed in advanced stage. As a result, prognosis is poor and the overall 5 year survival rate is only 30-40% despite proper treatment.[3]It is evident that the main cause for the release of tumor cells in invasive carcinomas is the loss of integrity of intercellular junctional molecule. As ovarian carcinomas show frequent metastasis involving the abdominal cavity, a close association has been observed between adhesion molecules and tumor advancement.[4]

CD56, also known as neural cell adhesion molecule (NCAM), is a cell surface glycoprotein that belongs to the immunoglobulin gene superfamily.[5] It has an extracellular portion composed of five Ig domains and two fibronectin type-III repeats. It is primarily expressed in nervous system, neuroendocrine, natural killer, and T cell lineages.[6,7]It regulates adhesion between neurons and between muscle and neurons. It plays important functional roles during neurite outgrowth and neuronal migration and immune surveillance. CD56 mediates these activities by both homophilic and heterophilic interactions.[6] Aberrant CD56 expression is seen in a variety of hematological malignancies such as multiple myeloma, myelocytic and lymphocytic leukemia[8] as well as solid tumors such as small cell lung cancer[9], Merkel cell cancer[10] and ovarian cancer.[11]CD56 is expressed in altered ovarian epithelium where it interacts with FGFR and stimulates it. Aberrant FGFR signaling causes tumor cell migration and invasion.[12]

Various studies showed that CD56 has significant association with high grade and advanced tumor stage in epithelial ovarian carcinomas.[3,4,12,13] Some studies also documented CD56 as an independent prognostic marker.[3,13] Recently, a study has been conducted on small cell lung cancer xenograft models and it showed high efficacy of a new CD56 antibody-drug conjugate.[14] This assures the possibility of CD56 being a therapeutic target in EOC in near future. Besides, CD56 may serve as a novel prognostic marker of EOC. However, only limited numbers of studies have been done on the pattern of expression of CD56 in epithelial ovarian carcinomas.

The aim of the study was to investigate the pattern of expression of CD56 in EOC and correlate with histopathological grading and staging.

Methods

This cross sectional study was carried out in the Department of Pathology, Dhaka Medical College, Dhaka from March 2018 to February 2020 which enrolled fifty histopathologically diagnosed cases of epithelial ovarian carcinomas. During the collection of specimen, all relevant information were recorded systematically in a predesigned data sheet. All the cases were numbered chronologically and the same number was given to histological as well as in immunohistochemical slides. Resected specimens were collected and preserved in 10% buffered neutral formalin solution at room temperature for 24 hours. Histopathological typing and grading were done according to WHO recommendation. The spread of tumor was classified according to TNM staging system.

For immunohistochemistry staining, 4-micrometer thick tissue sections were taken on poly-L lysine coated slide from the paraffin blocks of tumor. Flex Monoclonal Mouse Anti-Human CD56, Clone 123C3, Ready- to- Use, (Dako Autostainer/Autostainer Plus), Code IS628, Glostrup, Denmark was used as primary antibody. Envision (Ready- to- Use, Dako) was used for CD56 assecondary antibody.

For the semiquantitative scoring of CD56 Remmele/Stegnerimmunoreactive score (IRS score) was used. Based on the staining of the tumor tissue, the intensity of cytoplasmic membranous immunoreactivity was graded from 0 to 3, with 0 as no color reaction, grade 1 as mild reaction, grade 2 as moderate reaction and grade 3 as intense reaction. The score was obtained by multiplying this grade with a factor determined by the percentage of positive tumor cells (No positive cells=0, <10%=1; 10-50%=2; 51-80%=3; >80%=4). Score 9- 12 was considered strongly, score 4-8 moderately, and score 2- 3 mildly positive. Score 0-1 was considered negative.[15]

Results

In our study fifty cases of epithelial ovarian carcinoma were included. Age of the patients ranged from 21 to 70 years. Most of the patients (38%) were in fourth decade. Mean age was 45.96 ± (11.47) years (Table I).

In this study we found four histological subtypes containing 34 (68%) serous, 9 (18%) mucinous, 5 (10%) endometrioid and 2 (4%) clear cell carcinoma (Table III). Most of the cases presented with high grade (Grade 2, 3) and advanced stage (Stage 2, 3) of disease.

Table I: Distribution of the study population according to age (n=50)

Age (Years)	Frequency	Percentage
21-30	5	10.0
31-40	10	22.0
41-50	19	38.0
51-60	12	22.0
61-70	4	8.0
Total	50	100.0

Mean ± SD (Min-Max) 45.96 ± 11.47 (21-70)

Immunohistochemical expression of CD56 was evaluated by using a semiquantitative score which categorized the immunoreactive cases into mild, moderate and strongly positive cases (Fig-1). Out of 50 cases, 17 (34%) cases were positive for CD56 which were further subdivided into 5 (10%) mild, 11 (22%) moderate and 1 (2%) strongly positive cases (Table II).

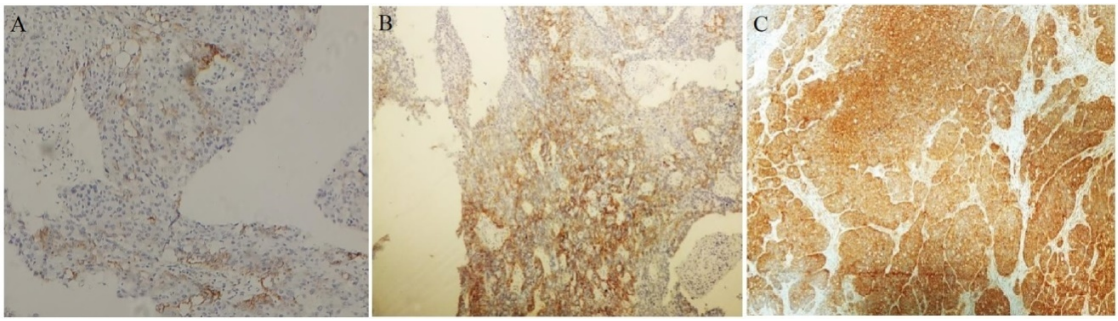

Figure 1. Immunohistochemical staining of serous carcinoma (A) CD56 IRS 2 100x (B) CD56 IRS 6 100x (C) CD56 IRS 12 100x

Serous carcinoma showed the highest number of CD56 immunoreactivity (82.4%). We also observed that positive expression of CD56 had significant association with high grade (P=0.041) and advanced stage (P=0.015) of epithelial ovarian carcinomas (Table III). Only 2 tumors in grade 1 (66.7%), 5 tumors in grade 2 (71.4%) and 4 tumors in grade 3 (57.1%) showed at least moderate staining. Only one tumor in grade 3 (14.3%) showed strong staining. In case of staging only 1 tumor in stage 1 (50%), 2 tumors in stage 2 (66.7%) and 8 tumors in stage 3 (66.7%) showed at least moderate staining. Only one tumor in stage 3 (8.3%) showed strong staining (Table III).

Table II: Distribution of the study population according to CD56 expression (n=50)

CD56 expression	Number of patients	Percentage
Positive	17	34.0
○ Mild	5	10
○ Moderate	11	22
○ Strong	1	2
Negative	33	66.0

Table III: Distribution of the study population according to variables with CD56 expression (n=50)

variables	CD56 expression Positive (n=17) n(%)	Negative (n=33) n(%)	P value	CD56 expression Mild (n=5)	Moderate (n=11)	Strong (n=1)	P value
Age (in years)							
Mean ± SD	48.12± 8.70	44.85 ± 12.65	0.345				
Median	48.0	44.0					
Histological diagnosis							
Serous carcinoma	14(82.4%)	20(60.6%)	0.118	5(35.71%)	8(57.14%)	1(7.14%)	0.370
Mucinous carcinoma	1(5.9%)	8(24.2%)	0.141	0	1(100%)	0	0.748
Endometrioid carcinoma	2(11.8%)	3(9.1%)	0.999	0	2(100%)	0	0.539
Clear cell carcinoma	0	2(6.1%)	0.542	0	0	0	
WHO grading							
Grade 1	3(17.6%)	14(42.4%)		1(33.3%)	2(66.7%)	0	
Grade 2	7(41.2%)	15(45.5%)	0.041	2(28.6%)	5(71.4%)	0	0.816
Grade 3	7(41.2%)	4(12.1%)		2(28.6%)	4(57.1%)	1(14.3%)	
TNM Staging							
T1	2(11.8%)	16(48.5%)		1(50%)	1(50%)	0	
T2	3(17.6%)	7(21.2%)	0.015	1(33.3%)	2(66.7%)	0	0.928
T3	12(70.6%)	10(30.3%)		3(25%)	8(66.7%)	1(8.3%)	

p value obtained by Chi-square test

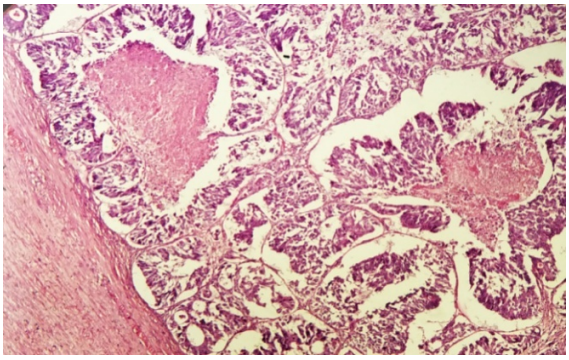

Figure 2. Photomicrograph showing a case of grade 2 serous adenocarcinoma (H&E X 100)

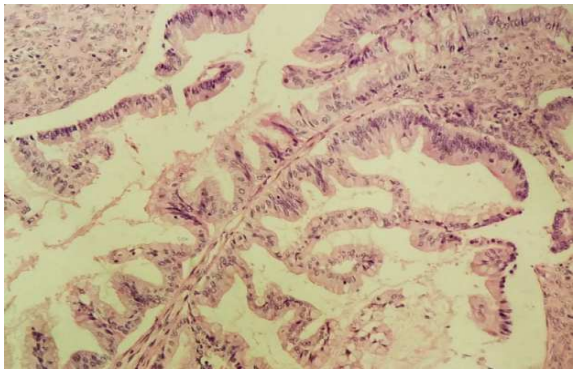

Figure 3. Photomicrograph showing a case of grade 1 mucinousadenocarcinoma (H&E X 200)

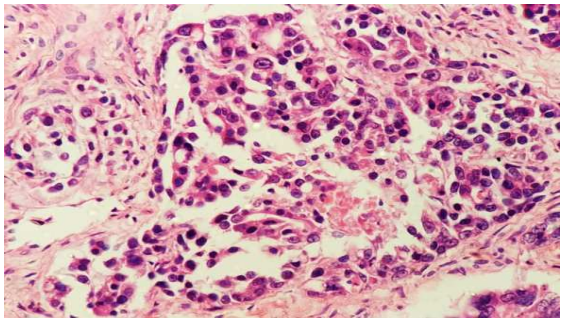

Figure 4. Photomicrograph showing a case of grade 2 endometrioid carcinoma (H&E X 400)

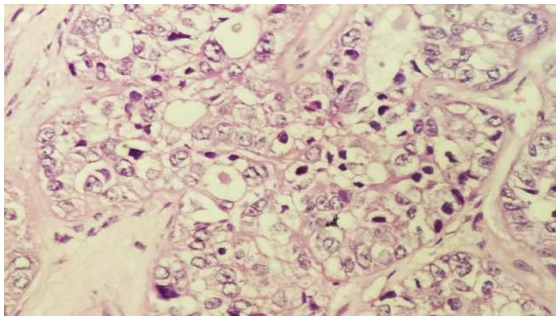

Figure 5. Photomicrograph showing a case of grade 2 clear cell carcinoma (H&E X 400)

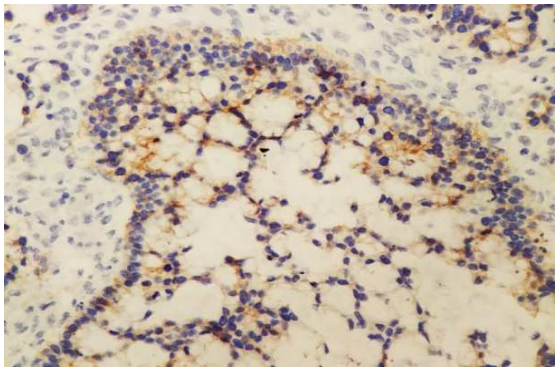

Figure 6. Photomicrograph showing CD56 expression (score: 6) in grade 2 mucinous adenocarcinoma (IHC X 400)

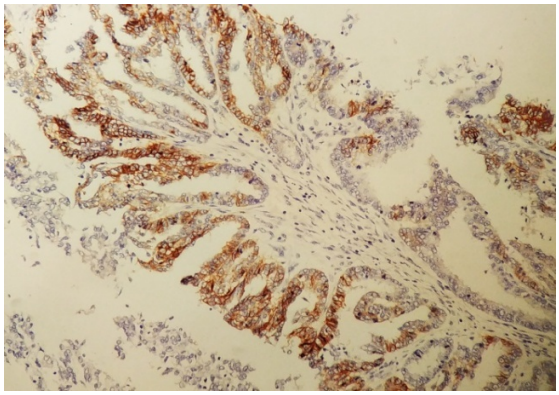

Figure 7. Photomicrograph showing CD56 expression (score: 6) in grade 1 serous adenocarcinoma(IHC X 200)

Discussion

Epithelial ovarian carcinoma comprises 90% of ovarian malignancy which in most of the cases presents with advanced stage that leads to poor survival. In metastatic ovarian carcinoma, a close relationship has been found between adhesion molecules and tumor progression.[4] CD56 is an adhesion molecule that belongs to immunoglobulin gene superfamily. It has been documented that variable degree of CD56 expression occurs in different types of ovarian carcinomas.[3]

This cross sectional study was undertaken in a tertiary care hospital in Bangladesh to inquire into the pattern of expression of CD56 in EOC and correlate with histopathological grading & staging. A total 50 histopathologically diagnosed cases of EOC of any age group were included in this study. Patients with recurrent EOC and patients who received chemotherapy prior to surgery were excluded from the study.

Here in this study, we found that the age of the patients ranged from 21 to 70 years. Most of the cases (38.0%) were found to be in age group 41– 50 years (median =45). The mean age of the patients was 45.96 ± (11.47) years (Table I). The result is almost similar to the study done by Mondal et al. (2011), in India who reported that median age of presentation of ovarian malignancy was 48 years and maximum cases were in age group 41-50 years.[16] In another study, Janagam & Atla (2017) showed that most of the malignant ovarian tumors occurred above the age of 40 years.[17] The current study showed that patients from 41 to 50 years of age group were mostly positive for CD56 (47.1%). There was no significant association between age and CD56 expressions (Table III). Davidson et al., (2015) found in their study that mean age of patients was 63 years and it was unrelated to CD56 expression. .[13] But Bosmuller et al. (2017) observed that median age was 68 years and there was a significant association between age and CD56 expression.[3]

We found four histological subtypes in our study. The most common subtype was serous carcinoma which was found to be in 34 (68%) cases followed by mucinous carcinoma which was in 9 (18%) cases. The other subtypes were endometrioid carcinoma and clear cell carcinoma which were present in 5 (10%) and 2 (4%) cases respectively (Table III). Bosmuller et al. (2017) found in their study that among 206 cases 76.69% of cases were serous carcinoma, 15.53% of cases were endometrioid carcinoma, 2.42% of cases were mucinous carcinoma and 5.33% of cases were clear cell carcinoma which is almost similar to our findings.[3] In another study, Deeba et al. (2013) observed serous carcinoma in 57.1% of cases, mucinous carcinoma in 17.9% of cases and endometrioid carcinoma in 3.6% of cases.[18]

The current study showed that out of 50 cases, 17 (34%) cases were positive for CD56. Among CD56 positive cases, 11 (22%) cases were moderately positive, 5 (10%) cases were weakly positive and only 1 (2%) case was strongly positive (Table II).Among 252 cases, Zecchini et al. (2011) observed positive CD56 expression in 60 (23.8%) cases.[12]Bosmuller et al. (2017) noticed positive CD56 expression in 65% cases.[3]

In this study, among 34 serous carcinoma cases, CD56 was positive in 14 (82.4%) cases. In case of 9 mucinous carcinomas, CD56 was positive in only 1 (5.9%) case. Among 5 endometrioid carcinomas CD56 was positive in 2 (11.8%) cases . No clear cell carcinoma cases stained positive for CD56 (Table III).Therefore, although number of cases varied in each subtype, maximum CD56 positive cases were found to be in serous

carcinoma. This finding supports the study done by Bosmuller et al. (2017).[3]

In this study, majority of the cases were moderately differentiated (grade 2) which were found to be in 22 (44%) cases. Grade 1 was present in 17 (34%) cases and grade 3 was present in 11 (22%) cases (Table III). Akhter et al. (2019) in Bangladesh reported that most of the malignant ovarian tumor (74%) were in grade 2 at the time of diagnosis.[19] But other studies noted that maximum ovarian malignancy presented with grade 3.[3,12]

Considering CD56 expression with WHO grading, the current study revealed that 7 (41.2%) positive cases were in grade 3 and 7 (41.2%) positive cases were in grade 2. The remainder 3 cases were in grade 1 accounting for 17.6%. On statistical analysis this difference was found to be significant (p= 0.041) (Table III). Bosmuller et al. (2017) observed that CD56 was significantly associated with high grade EOC especially in high grade serous carcinoma.[3] Similar findings were reported by Zecchini et al. (2011) and Cho et al. (2006).[12,4]

In present study we also observed that in grade 1, 1 (33.3%) case showed mild CD56 expression, 2 (66.7%) cases showed moderate CD56 expression with none of the cases showing strong CD56 expression. In grade 2, among 7 cases 2 (28.6%) cases showed mild CD56 expression and 5 (71.4%) cases showed moderate CD56 expression. In grade 3, among 7 cases 2 (28.6%) cases showed mild CD56 expression, 4 (57.1%) cases showed moderate CD56 expression and 1 (14.3%) case showed strong CD56 expression (Table III). Thus, we noticed that there was a trend for increasing staining intensity with higher grade which however was not statistically significant (p>0.05). But Bosmuller et al. (2017) noted that there was a significant correlation between staining pattern of CD56 and grading.[3]

In present study maximum (44%) cases belonged to stage 3. Stage 1 was found to be in 18 (36%) cases and stage 2 was found to be in 10 (20%) cases. We observed that 12 (70.6%) CD56 positive cases were in stage 3, while 2 (11.8%) positive cases were in stage 1 and 3 (17.6%) positive cases were in stage 2. Therefore, stage 3 showed maximum number of CD56 positive cases than stage 1 and stage 2. The difference among three groups was statistically significant (p= 0.015) (Table III). This finding reflected some previous studies.[3,4] Some studies also reported that CD56 was an independent prognostic factor for patient survival.[3,13]

We also observed that in stage 1, one (50%) case showed mild CD56 expression and one (50%) case showed moderate CD56 expression with none of the cases showing strong CD56 expression. In stage 2, among 3 cases 1 (33.3%) case showed mild CD56 expression and 2 (66.7%) cases showed moderate CD56 expression. In stage 3, among 12 positive cases 3 (25%) cases showed mild CD56 expression, 8 (66.7%) cases showed moderate CD56 expression and 1 (8.3%) case showed strong CD56 expression (Table III). Thus, we noticed that there was a trend for increasing staining intensity in higher stage, although the difference did not reach statistical significance. However, Bosmuller et al. (2017) found that there was a significant correlation between staining pattern of CD56 and staging.[3]

Conclusion

The current study reveals that Bangladeshi patients present with epithelial ovarian carcinoma at an early age compared to western countries. Most of the tumors are in

high grade and advanced stage at the time of diagnosis. CD56 expression positively correlates with histopathological grading and staging. Although there is a trend for increasing staining intensity in higher grade and stage, the differences does not reach statistical significance. This may be due to smaller sample size. According to some previous studies CD56 expression has potential prognostic and therapeutic relevance which remains to be established. Therefore, further studies with larger sample size with patient follow up are recommended.

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