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# Tumour Infiltrating Lymphocytic (TIL) Response in Epithelial Ovarian Carcinoma and their Correlation with Histopathological Grading and Staging

Dr. Arebia Rahman[1*], Dr. Mahbuba Zhumur[2], Prof. Dr. Md. Rezaul Karim Dewan[3], Dr. Tahmina Sultana[4], Dr. Naila Awal[5], Dr. Mohammad Shahriar Rahman[6], Dr. Labiba Yasmin Rahman[7]

[1]Associate Consultant, Department of Pathology, Square Hospital Ltd.
[2]Assistant Professor of Pathology, Shaheed Suhrawardy Medical College
[3]Professor and Head, Department of Pathology, Green Life Medical College
[4]Lecturer, Department of Histopathology, National Institute of Cancer Research and Hospital, Dhaka, Bangladesh
[5]Associate Professor, Department of Pathology, Green Life Medical College
[6]Assistant Professor, Department of Orthopaedics, Dhaka Medical College
[7]Assistant Professor & Head (CC), Department of Pathology, Institute of Applied Health Sciences (IAHS), USTC Medical College, Chittagong

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***Corresponding author:** Dr. Arebia Rahman
Associate Consultant, Department of Pathology, Square Hospital Ltd.

**Abstract** | **Original Research Article**

***Background:*** Epithelial ovarian carcinoma (EOC) remains one of the most lethal gynecological malignancies, primarily due to late-stage diagnosis in the majority of patients. Although advancements in surgical techniques and chemotherapy have improved treatment strategies, overall survival rates have seen minimal improvement. Recent research highlights the critical role of the immune system in tumor progression, particularly the prognostic significance of tumor-infiltrating lymphocytes (TILs), such as CD3+ and CD8+ T cells. These immune cells are associated with enhanced anti-tumor responses and improved patient outcomes, suggesting their potential as both biomarkers and therapeutic targets in EOC. *Objectives:* The purpose of the present study was to detect the CD3 and CD8 positive TIL in EOC & their correlation with grading and staging. ***Methodology:*** A cross-sectional observational study was conducted at Dhaka Medical College from March 2017 to June 2019, involving 50 histologically confirmed epithelial ovarian carcinoma cases. Tissue samples were processed for routine histopathology and immunohistochemical staining to detect CD3 and CD8 tumor-infiltrating lymphocytes. Relevant demographic and clinical data were recorded. Cases with prior chemotherapy or recurrence were excluded. ***Results:*** Immunohistochemical analysis revealed significant associations between CD3 and CD8 tumor-infiltrating lymphocytes (TILs) and epithelial ovarian carcinoma (p=0.001). Both TIL markers showed strong correlations with high-grade tumors (p=0.007) and advanced-stage disease, particularly in serous carcinoma. ***Conclusion:*** Epithelial ovarian carcinoma demonstrates immunological involvement, with CD3 and CD8 TILs significantly associated with high-grade and advanced-stage tumors, especially the serous subtype. TIL evaluation may serve as a valuable prognostic tool and aid in guiding patient management.

**Keywords:** Epithelial Ovarian Carcinoma (EOC), Tumor-Infiltrating Lymphocytes (TILs), CD3+ T cells, CD8+ T cells, Grading and Staging.



## INTRODUCTION

Ovarian cancer remains the most lethal of all gynecological malignancies, with the poorest prognosis among female reproductive system cancers [1]. According to the World Health Organization (WHO), ovarian neoplasms are categorized into three major histological subtypes based on their tissue of origin: (A) Surface epithelial tumors, (B) Sex-cord stromal tumors, and (C) Germ cell tumors [1]. Of these, surface epithelial-stromal tumors constitute approximately 60% of all ovarian tumors and nearly 90% of all ovarian malignancies [2].

Despite high initial response rates to standard chemotherapy, particularly platinum-based regimens, the long-term survival of patients with advanced epithelial ovarian carcinoma (EOC) remains dismal,

with fewer than 40% surviving beyond five years [3]. Clinical outcomes in EOC are influenced by tumor stage, the extent of residual disease following cytoreductive surgery, and the response to chemotherapy. However, significant variability in progression-free survival and overall survival has been observed even among patients with similar clinicopathological features [4,5].

Emerging evidence suggests that this variability may be partly explained by host immune response mechanisms. The interaction between the immune system and ovarian cancer cells has long been recognized, but only recently has attention turned to understanding how immune factors, particularly tumor-infiltrating lymphocytes (TILs), influence disease progression and patient outcomes [6].

TILs, especially CD3+ (pan-T cells) and CD8+ (cytotoxic T cells), have been associated with improved prognosis in ovarian cancer. Studies have demonstrated that the presence of these immune cells within the tumor microenvironment correlates with better clinical outcomes, including longer survival times and reduced recurrence (Zhang *et al*., 2003) [7]. In particular, CD8+ TILs are thought to mediate antitumor immunity and are considered independent prognostic indicators in EOC, especially in advanced stages.

Given this background, the present study was designed to evaluate the presence and significance of CD3 and CD8 TILs in epithelial ovarian carcinoma. Specifically, we aimed to assess the relationship between TIL expression and histological subtypes, tumor grades, and clinical stages of EOC, thereby providing further insight into the prognostic role of immune infiltration in this aggressive malignancy.

## Materials and Methods

This cross-sectional observational study was conducted in the Department of Pathology, Dhaka Medical College, from March 2017 to June 2019. A total of 50 histopathologically confirmed cases of epithelial ovarian carcinoma were included. Patients with prior exposure to neoadjuvant chemotherapy or with recurrent tumors were excluded. Clinical and demographic data were recorded in a structured proforma. Surgical specimens were fixed in 10% buffered formalin, processed by standard paraffin-embedding technique, and stained with hematoxylin and eosin. Tumors were graded and staged following WHO and TNM classification guidelines. Immunohistochemical staining for CD3 and CD8 was performed at the Armed Forces Institute of Pathology (AFIP), Dhaka, using 4-micron thick sections mounted on poly-L-lysine-coated slides. Positive TILs showed brown staining on the cytoplasmic membrane of lymphocytes.

Tumor-infiltrating lymphocytes (TILs) were evaluated manually in the most densely infiltrated intraepithelial areas across 15–20 high-power fields. The average count was scored as follows:

- Score 0: No positive cells (Negative)
- Score 1: ≤5 positive cells
- Score 2: 6–19 positive cells
- Score 3: ≥20 positive cells

Scores ≥1 were considered positive. Correlations between CD3/CD8 expression and tumor grade and stage were analyzed statistically.

## Results

**Table 1: Distribution of the study patients according to CD3 TIL expression (n=50)**

| CD3 TIL expression | Number of patients | Percentage | *p* value |
|---|---|---|---|
| **Positive** | **38** | **76.0** | |
| Score 1 | 5 | 10 | |
| Score 2 | 9 | 18 | **0.001[s]** |
| Score 3 | 24 | 48 | |
| **Negative** | **12** | **24.0** | |

**Table 1** shows CD3 Tumour infiltrating lymphocytes expression. More than three fourth of patients (76.0%) had positive CD3 TIL expression and 12 cases (24.0%) had negative CD3 TIL expression. The difference was found statistically significant ($p<0.05$).

**Table 2: Distribution of the study patients according to CD8 TIL expression (n=50)**

| CD8 TIL expression | Number of patients | Percentage | *p* value |
|---|---|---|---|
| **Positive** | **30** | **60.0** | |
| Score 1 | 9 | 18 | |
| Score 2 | 9 | 18 | **<0.05[s]** |
| Score 3 | 12 | 24 | |
| **Negative** | **20** | **40.0** | |

s= significant

**Table 2** shows the distribution of the study patients according to CD8 Tumour infiltrating lymphocytes expression. It was observed that two third of patients (60%) had positive CD8 TIL and 20 patients (40%) were negative for CD8 TIL. Which was found statistically significant ($p$<0.05).

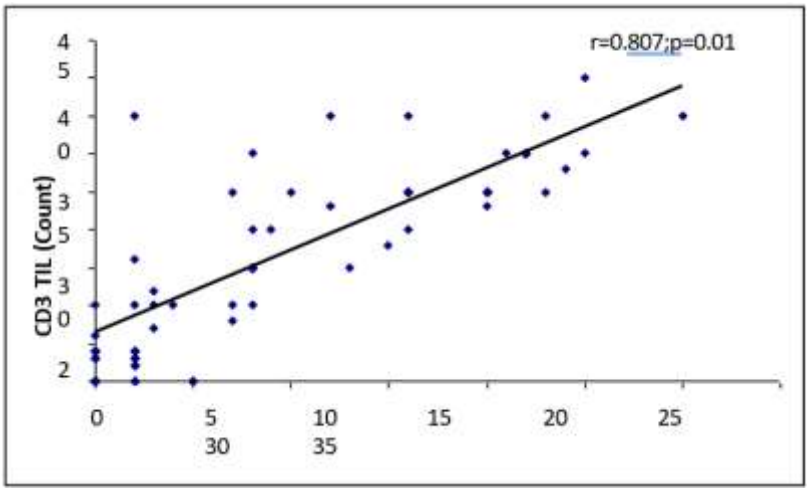


**Figure I: Correlation between CD3 TIL and CD8 TIL expression. (n=50)**
TIL= Tumour Infiltrating Lymphocytes

**Figure I:** The scatter diagram shows the relationship between CD3 Tumour Infiltrating Lymphocytes count and CD8 Tumour Infiltrating Lymphocytes count in epithelial ovarian carcinoma. In patients, who had increased CD3 count also had increased CD8 TIL count. It suggests a positive correlation (r =0.807; p=0.001) between CD3 TIL and CD8 TIL counts.

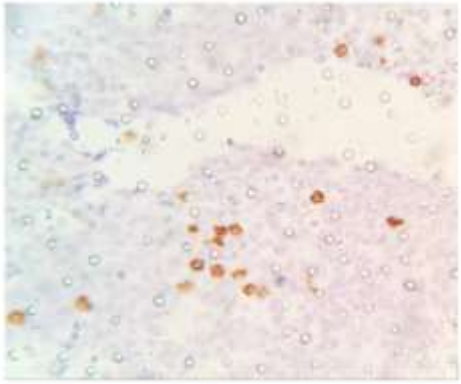

**Figure I: Photomicrograph showing a case of papillary serous cyst adenocarcinoma (Grade-3)**

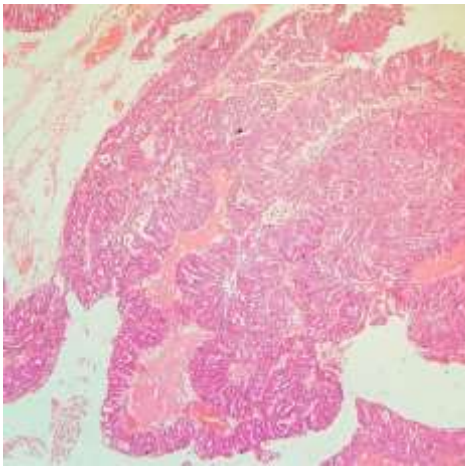

**Figure II: Photomicrograph showing high (score 3) CD3 positive lymphocyte infiltration**

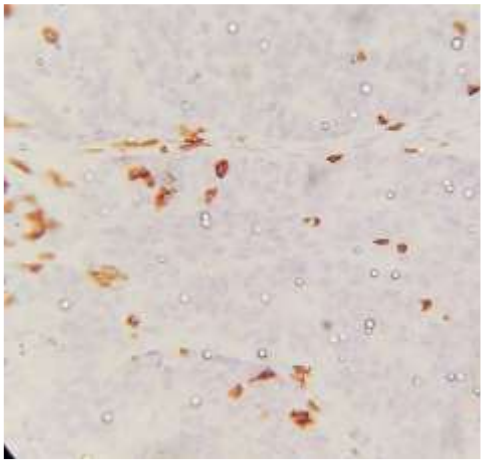

**Figure III: Photomicrograph showing score 2 CD8 positive lymphocyte infiltration**

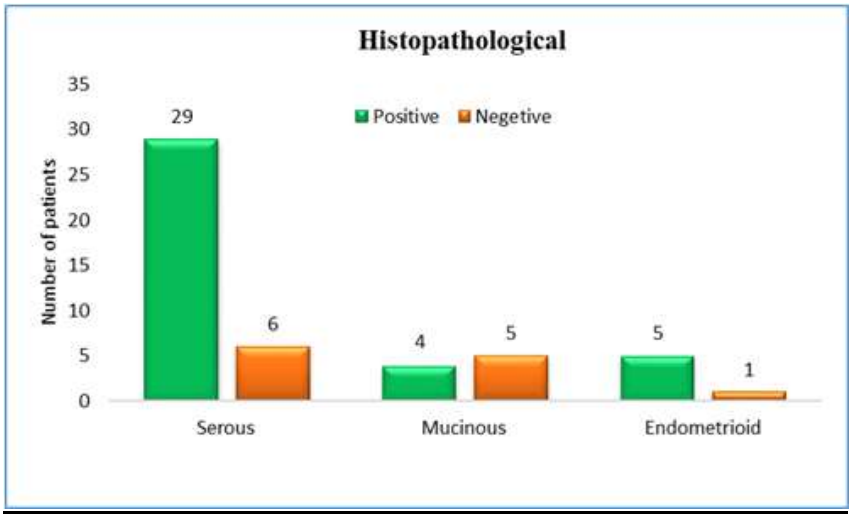


**Figure IV: Bar diagram showing CD3 TIL expression with histopathological type of EOC. (n=50)**

This Figure shows CD3 TIL was positive (82.9%) serous carcinomas cases (44.4%) Mucinous carcinoma cases and (83.3%) endometrioid carcinoma cases, maximum CD3 TIL positive cases were found in serous carcinoma.

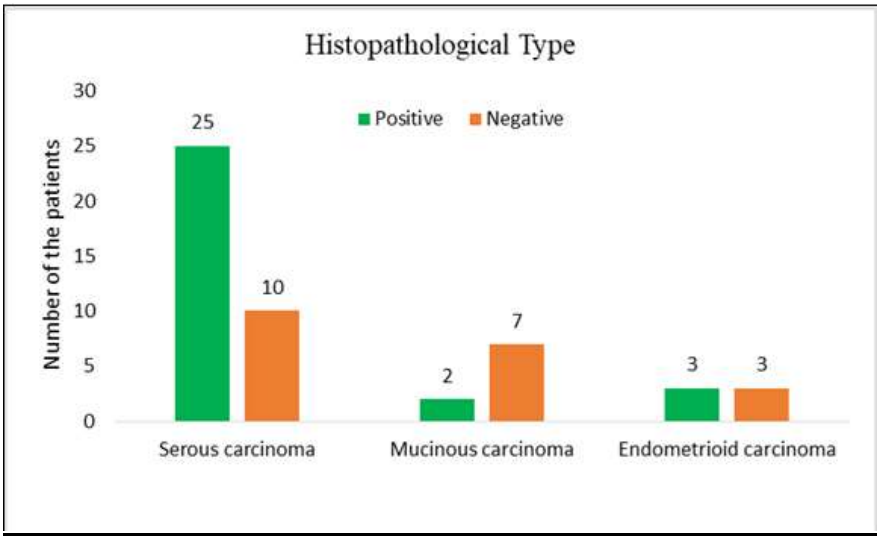


**Figure V: Bar diagram showing CD8 TIL expression with histopathological type of EOC. (n=50)**

This Figure shows among 35 serous carcinoma cases 25 cases (71.4%) CD8 TIL positive. Among 9 Mucinous carcinoma, two cases (22.2%) and among 6 endometrioid carcinoma three patients (50.0%) were found CD8 TIL positive.

**Table 4: Distribution of the study population according to CD3 TIL expression with WHO grading (n=50)**

| CD3 TIL expression | | | | | |
|---|---|---|---|---|---|
| **WHO grading** | **Positive** | | **Negative** | | ***p* value** |
| | **n** | **%** | **n** | **%** | |
| Grade 1 | 9 | 23.7 | 3 | 25.0 | |
| Grade 2 | 1 | 2.6 | 4 | 33.3 | 0.007[s] |
| Grade 3 | 28 | 73.7 | 5 | 41.7 | |
| **Total** | 38 | 100.0 | 12 | 100.0 | |

Table 4 shows the three fourth patients (73.7%) had positive CD3 TIL in grade 3, one patient (2.6%) was in grade 2 and nine patients (23.7%) were in grade 1. These differences were found statistically significant ($p<0.05$) among all three groups.

**Table 5: Distribution of the study population according to CD8 TIL expression with WHO grading (n=50)**

| CD8 TIL expression | | | | | |
|---|---|---|---|---|---|
| **WHO grading** | **Positive** | | **Negative** | | ***p* value** |
| | **n** | **%** | **n** | **%** | |
| Grade 1 | 4 | 13.3 | 8 | 40.0 | |
| Grade 2 | 1 | 3.3 | 4 | 20.0 | 0.006[s] |
| Grade 3 | 25 | 83.4 | 8 | 40.0 | |
| **Total** | 30 | 100.0 | 20 | 100.0 | |

**Table 5** shows CD8 Tumour infiltrating lymphocytes expression with WHO grade. Total number of CD8 positive TIL was 30 majority of the patients (83.4%) had positive CD8 TIL in grade 3, one patient (3.3%) was found in grade 2 and four patients (13.3%) were in grade 1. These differences were found statistically significant ($p<0.05$) among all three groups.

**Table 6: Distribution of the study patients according to CD3 TIL expression with TNM staging (n=50)**

| CD3 TIL expression | | | | | |
|---|---|---|---|---|---|
| **TNM Staging** | **Positive** | | **Negative** | | ***p* value** |
| | **n** | **%** | **n** | **%** | |
| T1 | 14 | 36.8 | 7 | 58.3 | |
| T2 | 2 | 5.3 | 3 | 25.0 | 0.020[s] |
| T3 | 22 | 57.9 | 2 | 16.7 | |
| **Total** | 38 | 100.0 | 12 | 100.0 | |

**Table 6** shows the distribution of the study population according to CD3 Tumour infiltrating lymphocytes expression with TNM staging. T3 stage showed maximum number of CD3 positive TIL cases than stage T1 and stage T2. The difference was found statistically significant ($p<0.05$) among three groups.

**Table 7: Distribution of the study patients according to CD8 TIL expression with TNM staging (n=50)**

| CD8 TIL expression | | | | | |
|---|---|---|---|---|---|
| **TNM Staging** | **Positive** | | **Negative** | | ***p* value** |
| | **n** | **%** | **n** | **%** | |
| T1 | 11 | 36.7 | 10 | 50.0 | |
| T2 | 1 | 3.3 | 4 | 20.0 | 0.048[s] |
| T3 | 18 | 60.0 | 6 | 30.0 | |
| **Total** | 30 | 100.0 | 20 | 100.0 | |

**Table 7** shows that almost two third (60%) of patients having positive CD8 TIL were in T3 stage, while one patient (3.3%) was in T2 and eleven patients (36.7%) were in T1 stage. The difference was found statistically significant ($p<0.05$) among three groups.

## DISCUSSION

Epithelial Ovarian Carcinoma (EOC) remains the most lethal gynecological malignancy, often diagnosed at an advanced stage due to its asymptomatic nature in early progression. The lack of a reliable

screening test has historically shifted the clinical focus toward treatment rather than prevention. Despite the use of debulking surgery and platinum-based chemotherapy, prognosis remains poor, with five-year survival rates in advanced stages (III & IV) ranging from only 18–47%.

Recent evidence highlights the prognostic relevance of tumor-infiltrating lymphocytes (TIL), particularly CD3+ and CD8+ subsets. This study evaluated the expression of CD3 and CD8 TIL in 50 primary EOC cases and found significant correlations between TIL presence, tumor grade, stage, and histological subtype.

A majority of cases (76%) showed CD3+ TIL positivity, comparable with findings by Sato *et al*. [8] (61%) and higher than Zhang *et al*. [7] (54.8%). Similarly, CD8+ TILs were detected in 60% of cases, aligning with previous studies, including Sato *et al*. [8] (58%) and Bachmayr-Heyda *et al*. [9] (100%). A strong positive correlation ($r = 0.807$, $p = 0.001$) was observed between CD3 and CD8 TIL expression, supporting prior reports by Han *et al*. [10] and Clarke *et al*. [11] who also documented concurrent expression in the majority of cases.

When analyzed by histological subtype, serous carcinoma showed the highest expression of both CD3 (82.9%) and CD8 (71.4%) TILs, consistent with earlier findings by Clarke *et al*. [11] and the OTTA consortium, which reported stronger TIL associations in serous compared to mucinous or endometrioid subtypes.

A significant relationship was also found between TIL presence and tumor grade. CD3+ and CD8+ TILs were predominantly observed in grade 3 tumors, with statistical significance ($p = 0.007$ and $p = 0.006$, respectively). These findings align with studies by Bosmuller *et al*. [**12]** and the OTTA consortium, suggesting a more pronounced immune response in high-grade tumors, although some earlier studies (e.g., Zhang *et al*. [7] Tomsova *et al*. [13] found no such correlation, possibly due to differences in sample size.

Moreover, staging analysis revealed that CD3 and CD8 TILs were more prevalent in advanced stages, particularly T3. CD3+ TILs were most commonly observed in stage T3 (57.9%), and CD8+ TILs also showed the highest expression in this stage ($p = 0.048$), indicating a significant correlation with disease advancement. These findings are supported by Clarke *et al*. and Bachmayr-Heyda *et al*. [9] who linked higher TIL infiltration with improved survival, particularly in advanced-stage serous carcinomas.

In summary, this study supports the prognostic significance of CD3 and CD8 TILs in EOC, particularly in high-grade and advanced-stage tumors, and reinforces the potential of immune profiling in guiding therapeutic strategies and prognostication.

## CONCLUSION

It can be concluded that Epithelial ovarian cancer is an immune-mediated tumour. Two-third of EOC cases of this study show positive TIL responses. Also, TIL had found significant correlation between high grade serous carcinoma and advanced stage of tumour. So, TIL response to ovarian cancer may serve as a novel prognostic marker. It might also be useful for implication of neoadjuvant chemotherapy and adaptive immunotherapy.

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