EVALUATION OF TUMOR INFILTRATING LYMPHOCYTES IN OVARIAN CARCINOMA AND ITS CORRELATION WITH VARIOUS SUBTYPES AND CARDING OF OVARIAN CARCINOMA

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"EVALUATION OF TUMOUR INFILTRATING LYMPHOCYTES IN OVARIAN CARCINOMA AND ITS CORRELATION WITH VARIOUS SUBTYPES AND GRADING OF OVARIAN CARCINOMA"

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ABSTRACT

INTRODUCTION

Ovarian cancer is one of the most common causes of cancer-related death among females. Surgery and chemotherapy can improve 5-year survival rate in approximately 45% of cases. Due to heterogeneous nature of ovarian carcinoma, evaluations of biomarkers which are prognostic factors are studied very rarely Tumour infiltrating lymphocytes (TILs) is one of the biomarkers considered as one of the prognostic factors for evaluating the survival of patients in various malignant tumours. However studies related to it are limited. Hence the present study was undertaken to evaluate the tumour-infiltrating lymphocytes in ovarian carcinoma and its correlation with various sub types and grading of ovarian carcinoma.

AIMS & OBJECTIVES OF THE STUDY

To determine the quantitative evaluation of TILs in ovarian carcinoma and to evaluate the association of TILs with sub types and grading of ovarian carcinoma.

MATERIAS AND METHODS

A combined retrospective and prospective study was done on 40 resected specimens of ovary which were diagnosed as ovarian carcinoma. These cases were evaluated for histopathological subtyping and grading of ovarian carcinoma. H& E stained sections were evaluated for TILs. These cases were further processed for IHC CD8 + T lymphocyte marker. Mean of TIL and CD8+ T lymphocyte expression was calculated on high power, by selecting random ten foci consisting of five intratumoral areas and five stromal areas with the highest TILs density. Also CD8+ T lymphocyte expression was evaluated. Correlation of the mean of TILs and CD8 + T lymphocytes was evaluated with histologic subtyping and grading of ovarian carcinoma.

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RESULTS

Total 40 cases were studied, out of which 33 cases were serous carcinoma, 5 cases were mucinous carcinoma and 2 cases were endometrioid carcinoma. Out of 33 cases of serous carcinoma, 18 cases were high grade and 15 cases were low grade. The coefficient of correlation between mean TILs and mean CD8+ TILs, was statistically significant with r=0.756 and are correlating significantly at the 0.01 level.

CONCLUSION

TILs density and CD8+ TILs showed a significant correlation and association with histopathological subtyping and grading of ovarian carcinoma. This suggest that TILs density and CD8+ TILs can be used as a prognostic biomarker, however multicentric study with more number of cases are needed for conclusion.

KEYWORDS

Tumour infiltrating lymphocytes (TILs), CD8+ T lymphocytes expression, ovarian carcinoma.

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LIST OF ABBREVATIONS:

TILs	Tumour infiltrating lymphocytes
WHO	World Health Organization
NK cells	Natural killer cells
EMA	Epithelial Membrane Antigen
ER	Estrogen receptor
PR	Progesterone receptor
FSH	Follicle stimulating hormone
LGSC	Low grade serous carcinoma
HGSC	High grade serous carcinoma
MMMTs	Malignant mixed mullerian tumours
Tregs	Regulatory T cells
TCR	T cell receptor
ADCC	Antibody-dependent cell-mediated cytotoxicity
TIL-B cells	Tumour-infiltrating B cells
TME	Tumour microenvironment

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INTRODUCTION:

Ovarian cancer is one of the most common causes of gynaecological cancer-related death among females. Surgery and chemotherapy in ovarian cancer can improve the 5-year survival rate of approximately 45% of the cases. Hence various authors thought that novel therapies are needed to increase the survival rate of these patients.[1]

Ovarian tumours are heterogeneous neoplasms having different outcomes. The overall incidence of epithelial tumours of the ovary varies from 9–17 per 1, 00,000 women.[2]

As per the World Health Organisation (WHO) classification, 65% of ovarian cancers are surface epithelial ovarian cancer. Surface epithelial ovarian tumours are classified as serous, mucinous, endometrioid, clear cell, and Brenner tumour.[3] Surface epithelial ovarian cancer is responsible for nearly 14,000 deaths per year in United States.[4]

In ovarian carcinoma, evaluations of biomarkers as prognostic factors are studied very rarely due to the heterogeneous nature of ovarian carcinoma. Tumour infiltrating lymphocytes (TILs) is one of the biomarkers considered in some studies as one of the prognostic factors for evaluating the survival of patients in malignant tumours. The studies related to the prognostic value of TILs in ovarian cancer are limited & the observations noted in these studies are controversial.[5]

Recent case studies have shown that the presence of TILs is considered as positive predictor of overall survival. These results suggest that host T cell immunity controls ovarian cancer progression. In some studies, it was mentioned that ovarian cancers are immunogenic tumours and can be recognized by the host immune system. It was also said that patients with T cell-rich tumours have longer progression-free and overall survival.[1,5]

TILs are generally white blood cells such as T lymphocytes, B lymphocytes, macrophages or natural killer cells (NK cells) that leave the blood and invade the tumour stroma or epithelium. TILs which infiltrate and accumulate tumour tissue are intratumoral and those located in adjacent stroma are called as peritumoral. Intraepithelial TILs in the immune system plays an essential role in controlling tumour growth in all solid tumours, mainly ovarian carcinoma. Well-established CD4+ and CD8+ T lymphocytes can recognize cancer antigens or overexpressed self-antigens and suppress cancer progression. Some tumour cells can interfere with recognition and response by the immune system. Recognizing the tumour

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in tissue by the endogenous immune system increases the inflammatory signals at the tumour site. Tumour-specific antigen-presenting cells can trigger T cell responses and the formation of cytokines such as “interferon gamma”. They promote local inflammation that completely destroys the tumour which is called as elimination. Tumours that survives elimination process enters in an equilibrium phase. Tumour cells rapidly mutate, and then become unstable and acquire resistance. Finally, the tumour enters an escape phase. During the escape phase, tumour cells evade recognition and killing by the immune system and eventually form metastatic tumours.[6]

Hence the present study was undertaken to evaluate the tumour-infiltrating lymphocytes in ovarian carcinoma and its correlation with various subtypes and grading of ovarian carcinoma.

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AIMS & OBJECTIVES OF THE STUDY:

1. To determine the quantitative evaluation of tumour infiltrating lymphocytes in ovarian carcinoma.
2. To evaluate the association of tumour infiltrating lymphocytes with subtypes and grading of ovarian carcinoma.

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REVIEW OF LITERATURE

ANATOMY & PHYSIOLOGY OF OVARY

The ovaries are paired pelvic organs located on the sides of the uterus close to the lateral pelvic wall, behind the broad ligament and anterior to the rectum. During pregnancy, the location and line of the ovary alter and rarely return to their original state. The tip of the examining finger can just reach the ovary in its usual position via the vagina. The ovary is made up of an outer cortex that houses the ovarian follicles and an inner vascular medulla that is surrounded by a layer of fibrous connective tissue called the tunica albuginea and coated by layer of cubical cells called the superficial epithelium. [7]

Ovarian surface epithelium, is the layer of modified mesothelium that covers the ovary. Epithelial Membrane Antigen (EMA), estrogen receptor (ER), Progesterone receptor (PR), CA125, follicle stimulating hormone (FSH), vimentin are immunoreactive for ovarian surface epithelium. In the cortex, epithelial inclusion glands and cysts are usually observed. Interestingly, the lining cells of the epithelial inclusion cysts are often PAX8 positive however ovarian surface epithelium is negative.[8]

At the time of birth, there are numerous primordial follicles under the ovarian capsule. Several of these follicles expand at the beginning of each cycle and forms a cavity around the ovum. In a human ovary, often on sixth day the follicles begins to grow rapidly and takes over as the dominant follicle, other follicles regress and develop into atretic follicles. One mechanism for the process of atrophy is apoptosis. It is not known exactly how follicles are selected as dominant follicles to secrete estrogen required for final maturation. In the ovaries granulosa cells are the main source of circulating oestrogen, but theca cells of the follicle are also essential for producing oestrogen because they release androgens and then converted to estrogen by the granulosa cells. The enlarged follicle ruptures, releasing the ovum cell into the peritoneal cavity on the 14th day of the cycle. The fimbriated ends of the uterine tubes pick up the ovum. It travels to the uterus and exits through the vagina if fertilization doesn't take place. When a follicle ruptures during ovulation, blood immediately fills the space referred to as a corpus hemorrhagicum. Further corpus luteum formation will be there. This is followed by proliferation of granulosa and theca cells which leads to initiation of luteal phase. Oestrogen and progesterone are secreted during this phase. If a pregnancy occurs and no menstrual cycle occurs until after delivery, the corpus luteum

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remains. In the absence of pregnancy, the corpus luteum starts to deteriorate four days before the next menstrual period and scar tissue finally replaces it, developing a corpus albicans.[9]

Ovarian cancer is one of the commonest malignancy in female and it is eighth common cause related to death in females worldwide.[10, 11] Incidence of ovarian malignancy varies in different geographic areas and different ethnic group. The highest incidence of ovarian cancer was observed in Northern Europe and United States, while Japan had the lowest prevalence. According to ethnic groups, the highest prevalence was noted in Caucasian women (12 per 100,000) followed by Hispanic (10.3 per 100,000) and Asian women (9.2 per 100,000). [13]

Nulliparity and mutation in tumour suppressor gene are the major risk factor in ovarian carcinoma. Incidence of cancer seen is high in both married and unmarried women with fewer pregnancies. Risk of malignancy was low in females who are on long term use of oral contraceptives.[12]

Approximately 5% to 10% of ovarian cancers are familial and most of them are associated with mutations in the BRCA1 and BRCA2 tumour suppressor genes. The average lifetime risk of ovarian cancer for BRCA1 carriers is approximately 30%. BRCA2 carriers are at slightly lower risk. BRCA1 and BRCA2 mutations are found in only 8% to 10% of sporadic ovarian cancers and may arise from different molecular mechanisms.[10]

Classification

Classification of ovarian tumours is primarily morphology of the tumour cells. Recent molecular studies support this morphology-based classification system, which accurately reflects both the cells of histogenesis /origin and also based on the molecular abnormalities underlying various ovarian tumour subtypes.[14]

As per the 2020 WHO classification of ovarian carcinoma is classified as[15]

- Serous carcinoma
    - Low grade serous carcinoma
    - High grade serous carcinoma
- Mucinous carcinoma
- Endometrioid carcinoma
- Clear cell carcinoma

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- Seromucinous carcinoma
- Malignant Brenner tumours
- Other carcinomas
    - Mesonephric-like adenocarcinoma
    - Undifferentiated and dedifferentiated carcinoma
    - Carcinosarcoma
    - Mixed carcinoma

Miscellaneous tumours

- Rete adenocarcinoma
- Small cell carcinoma of the ovary, hypercalcemic type

Epithelial tumours, have traditionally been thought to derive from the surface epithelium of ovary and were thus referred to as "surface epithelial" tumours. Surface epithelium becomes trapped in the ovarian cortex as a result of repeated ovulation and scarring, resulting in tiny epithelial cysts. These can be metaplastic or neoplastically change into a variety of epithelial tumours.[7] Ovarian surface epithelial tumours are classified based on the type of lining epithelium such as serous, mucinous, endometrioid and transitional or brenner tumour.[5,6]

Serous tumours account for 20% to 50% of ovarian tumours and are serous neoplasms. 70% to 75% of all ovarian carcinomas worldwide are invasive serous carcinomas.[16]

Serous carcinoma can arise as denovo or it may originate from benign or borderline tumour. Grading in serous carcinoma was done as LGSC and HGSC. LGSC is associated with mutations in KRAS, BRAF, or ERBB2. HGSC grow rapidly and they arise from tubal intraepithelial carcinoma rather than the ovarian coelomic epithelium. In LGSC proliferative index of Ki-67 is lower when compared to HGSC. Aberrant expression of p53 can be useful in diagnosis of HGSC. In recent studies it was noted that in deep sequencing in 96% of cases TP53 mutation was noted.[6]

Mucinous neoplasms are less common compared to serous neoplasms. They are usually bilateral. Mucinous ovarian tumours are categorised into benign, borderline and malignant. Most of the mucinous tumours are benign or borderline tumours. Mucinous

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carcinoma accounts for 3 to 4 % of all primary ovarian carcinomas.[14] In mucinous carcinoma histological grading is done as: Grade 1-no solid areas, Grade 2-up to 50% solid foci, Grade 3-more than 50% solid foci. While severe nuclear atypia is noted microscopically, grade I or II carcinomas increases by one grade. In mucinous carcinoma, CK7 expression is diffuse positive but CK20 expression is variable, it is usually less extensive than CK7 expression. PAX8 is typically expressed in 50-60% of tumours.[16]

Endometrioid tumours are derived from pre-existing endometriosis or from benign or borderline adenofibromas. Hyperestrogenic states appear to have a role in the malignant transformation of endometriosis. Endometrioid carcinomas, which account for up to 10% of ovarian cancers, are defined as neoplasms closely resembling endometrial endometrioid adenocarcinomas**.** These tumours can be solid or cystic and are associated with endometriosis. In 30% of cases, endometrioid tumours are bilateral. In 15% to 30% of cases of endometrioid tumours association with endometrial cancer was noted.[16] PTEN tumour suppressor gene mutations are found in endometrioid carcinomas of the ovary.[10]

Grading of endometrioid carcinoma of ovary can be done as per the FIGO criteria which is used for grading endometrioid carcinomas of the endometrium. More than 90% of ovarian endometrioid carcinomas are low-grade either grade 1 or 2.[16]

FIGO Histological grading was done as follows.

Grade 1: less than 5% solid component
Grade 2: 6 - 50% solid component
Grade 3: more than 50% solid component
Immunohistochemistry - EMA, CK7, PAX8 (84% positive), ER and PR (86% and 72% positive).

Clear cell carcinoma accounts for 10% of ovarian carcinomas. These tumours are associated with ovarian and pelvic endometriosis more frequently than any other epithelial–stromal tumour, including endometrioid carcinoma. These tumours are subdivided into benign, borderline and malignant categories. Clear cell malignant tumour (Clear cell carcinoma): Malignant clear cell tumours are usually graded as high grade clear carcinoma. This tumour consists of transparent, eosinophils and hobnail cells show a combination of tubular cystic, papillary and solid patterns. There is no well-validated grading system for clear cell carcinoma but they are graded as high grade. Clear cell carcinoma has glycogen-

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rich cytoplasm of clear cells show PAS-positive and diastase-sensitive. Sometimes diastase-resistant material can be seen in the apical cell membrane, Clear cell carcinoma shows 99% positivity with PAX8 and 12% cases are aberrant in p53 mutations. [16]

Seromucinous is a malignant tumour composed primarily of serous and endocervical mucinous epithelium.These tumours are extremely uncommon and can be seen at the age of 45 years. The mitotic activity varies but low (< 5 mf /10 HPF). These tumours usually express CK7 (positivity). In these tumours ER and PR positivity is noted. Majority of the seromucinous carcinoma are WT 1 negative. [16]

Brenner's tumour is a rare, solid, mostly unilateral ovarian tumour rich in stroma with foci of transitional epithelium similar to the urinary tract. Malignant brenner tumour is characterized by stromal invasion with tumour cells showing features of transitional epithelial and irregular nests of cells in an infiltrative pattern. Squamous or mucinous differentiation may be present. Malignant brenner tumour shows p63 and GATA3 positivity. [16]

Carcinosarcomas (MMMTs) of the ovary are classified as endometrioid because they resemble the tumours with those names that are most commonly found in the endometrium. These tumours have both epithelial and mesenchymal components. Carcinosarcomas are highly malignant tumours that most commonly develop in postmenopausal women.[16]

Mesodermal (müllerian) adenosarcomas, of which more than 50 cases have been reported, are mostly solid tumours containing numerous small cysts. They are seen in elder women with mean age of 54 years and have worse prognosis.[16]

Sex cord stromal tumours, which comprise approximately 5.4 % of all ovarian tumours. The most common subtype of sex cord stromal tumour is fibroma. The remainder exhibit differentiation toward one or more of the following cell types: granulosa cells (most frequent), theca cells, and Sertoli or Leydig cells (least common). A few tumours are intermediate or indifferent in their differentiation or contain two or more cell types. These tumours occur in combination and exhibits wide range of differentiation, which often repeats the patterns produced during embryogenesis of ovary and testis. [16]

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Recently, a number of immunohistochemical markers have become available that are of considerable assistance in the identification of these tumours. Inhibin has emerged as one of the most useful. The α-subunit of the molecule seems to have a greater degree of specificity than the β-subunit. It stains all types of sex cord– stromal tumours, the sex-cordlike elements of other gynecologic neoplasms, and most trophoblastic tumours. It is also useful for the identification of steroid hormone-secreting cells in the nonneoplastic stroma component of epithelial, germ cell, and other types of ovarian tumour. [16]

Fibromas are usually common in 4th decade. They are rarely seen in children, except in patients with autosomal dominant disorders called as Gorlin's syndrome. They are associated with elevated CA-125, which increases the likelihood of ovarian cancer, but are rarely associated with clinical or pathological signs of hormone production. When fibromas are greater than 10 cm in diameter, they can be associated with ascites. In Fibromas inhibin, calretinin and other IHC markers such as WT1 and SF1 are immunoreactive. [16]

Thecomas are associated with estrogenic symptoms, often manifested only by pathological conditions such as endometrial hyperplasia. Androgenic symptoms are rare. Thecomas are usually benign with rare exceptions. They may show malignant features with nuclear atypia and increased mitotic activity and can metastasize. Most of the thecomas are immunoreactive for inhibin, calretinin.[16]

Signet ring stromal tumour are rare neoplasm seen in adults. It has a solid or solid and cystic cut surface. Tumor cells may contain keratin and smooth muscle actin, but are negative for EMA, desmin, inhibin, calretinin, SF-1 and S100. In electron microscopic examination, the vacuoles may be caused by generalised cytoplasmic edema, mitochondrial swelling, or the development of cytoplasmic pseudoinclusions formed of edematous extracellular matrix.[16]

Steroid Cell Tumours account for approximately 0.1% of all ovarian tumours. They occur in a wide age range, but the median age (43 years) and half of the patients present with androgenic symptoms. Steroid Cell Tumours shows positivity for calretinin, inhibin, and steroidogenic factor-1 and are usually Melan-A 845 positive. [16]

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Germ cell tumours are approximately 20% of all ovarian neoplasms and seen in children and young adults, and approximately 95% are benign cystic teratomas. In young patients, germ cell tumours can be malignant.[14]

TUMOUR INFILTRATING LYMPHOCYTES

Tumour-infiltrating lymphocytes (TILs) are a distinct histological feature of several cancers that are thought to reflect an individual immunological tumour response. [17] Tumor-infiltrating lymphocytes are defined as the specific killing lymphocytes in the tumour microenvironment. [20]

TILs are white blood cells that have left the vasculature and stay in the tumour stroma or intraepithelium, such as T-cells, B-cells, macrophages, or natural killer cells. TILs are categorised as intratumoural and peritumoral lymphocytes. Intratumoural lymphocytes are the lymphocytes which invade tumour islands and the lymphocytes that are located in the adjacent stroma are called as peritumoral lymphocytes. Intraepithelial TILs play an important role in controlling tumour proliferation. [21]

Some cancer cells can interfere with T lymphocyte immune recognition and response. When the body's immune system recognizes a tumour growing in tissue, it amplifies inflammatory signals at the site. Tumor-specific antigen-presenting cells initiate T-cell responses and cytokines such as interferon gamma promote local inflammation leads to tumour destruction process known as elimination. Tumours that survive elimination enter into a state of equilibrium. Ultimately, the tumour enters an escape phase, evading detection by the immune system and becoming malignant, ultimately leading to metastasis. This immune infiltration is often a heterogeneous mixture of immune cells, including cells from both innate and adaptive immune populations. B-cell types associated with immune function, active (ex: cytotoxic T lymphocytes) and suppressive (ex: regulatory T cells, myeloid suppressor cells). TIL responses vary by tumour. Shows beneficial prognostic effects in some malignancies but adverse effects in other cancer types.[22]

According to Lin et al.[20] hypothesized that tumour infiltrating lymphocytes were seen due to autoimmune response to the tumour. TILs has breakthroughs with the technology advance, particularly the emergence of deep learning, artificial intelligence and recombinant genetic engineering products.

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Rosenberg et al.[36] isolated lymphocytes and transfused them in vitro with IL-2 amplification, which effectively controlled tumour growth in distant metastases to liver and lung. Metastatic and primary tumours vanished completely in some mice. Based on these findings they concluded that TIL has a remarkable curative effect. This is a significant advancement in TIL therapy.[20]

The T cell receptor (TCR) subunit and primary lineage markers CD8 and CD4 are commonly used to sort T cells. The TCR complex (CD4 T cells) allows T cells to recognize peptides present on the cell surface of MHC class I or MHC class II. TCR components sets to function primarily in MHC classes I and II. CD8+ and CD4+ TCR T cells are the most common T cell subsets in tissues, including tumour tissue.[25]

The presence of B lymphocytes is associated with improved prognosis such as hepatocellular carcinoma, breast cancer, colorectal cancer, renal cell carcinoma and head and neck squamous cell carcinoma. This may be due to B lymphocytes, which play an important role in immunotherapy. Functionally, B cells may serve as APCs for T cells, increasing regional T cell responses to tumours. The discovery of clonal expansion of B cells and altered immunoglobulin phenotypes in many human malignancies supports the role of antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by antibody-secreting plasma B cells in anti-tumour humoral immune responses. It also suggests possible roles. Tumour-infiltrating B (TIL-B) cells enter the tumour either indirectly by inducing the release of immunostimulatory cytokines by tumour-specific T cells or directly by secreting toxic cytokines such as IFN and granzyme B.[25]

The presence of TILs in tumours is associated with better prognosis. On the other hand, the type and function of TILs and the TME localization of different TILs are important for tumour suppression or progression. As a result, a more detailed examination of TIL spatial organisation in the tumour microenvironment (TME), such as marginal zone vs tumour stroma is required for better insight of antitumour activity.[25]

Tumour cells in stroma contains both immune cell components like B lymphocytes, T lymphocytes, macrophages, DCs, NK cells and non-immune cell components supporting TME. The stroma that surrounds the tumour cells is an important component of the TME because it contains both a cellular immune component and a nonimmune cellular component (B lymphocytes, T lymphocytes, NK cells, fibroblasts, endothelial cells and mesenchymal cells). Physiologically, stromal cells are suppressive, controlling the proliferation and

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migration of differentiated epithelial cells and also maintaining organ architecture and size. Immunologically active cytokines such as growth factors, chemokines, angiogenic factors, and interferons, play a significant role in tumour-stroma interactions. By secreting cytokines, stromal TILs serve as important immunological organizers in TME. CXCL13 is a well-known important chemokine in the structural organization of immune cell clusters. This allows CXCR5-expressing B and T cells to invade boundary and form well-organized structures. The body's first line of defence against cancer metastasis is the invasion margin.[6]

On light microscopy, the semi quantitative evaluation of H&E-stained slides is the most commonly used method for detecting TILs. Intratumoral TILs are rare and therefore difficult to recognize and evaluate. Stromal TILs are found in the stroma between tumour cell clusters. They are common and numerous. TILs have been shown to be a good prognostic factor in solid tumours. TILs have been shown to improve survival in ovarian cancer patients. Many significant studies have been conducted in recent years.[27, 28]

A study by Coukos et al.[1] examined 186 advanced-stage ovarian cancer samples. They evaluated the patients with detectable CD3 intraepithelial TILs had a 5-year survival rate of 38%, compared with only 4.5% of those without TILs. Including the patient who achieved good clinical response after dose reduction and chemotherapy, the improvement in 5-year survival increased to 73.9% in cases without TILs.

Although a study by pinto et al. [28] has observed that CD3+ TILs survival improvement in patients with high CD3 TILs. Further studies point to CD8+ TILs as the cells responsible for improved survival in ovarian cancer.

Study by sato et al. [32] found that intraepithelial CD8+ TILs improved survival, but did not see an association with CD3+ TILs. This study has shown that CD8+ TILs maintains favourable prognosis in ovarian cancer. Although CD8+ TILs appear to be most strongly associated with improved survival.

Other studies suggested that CD4+ TILs are the most useful predictors of ovarian cancer survival. Some studies have shown negative or no correlation between TILs and survival, the presence of TILs is associated with a positive prognosis in ovarian cancer.[21]

Hwang et al.[23] conducted A recent meta-analysis evaluating the prognostic value of TILs in ovarian cancer and examining other prognostic factors such as survival. They found 10 of his studies on malignant ovarian tumours to be included in the meta-analysis. 1815

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subjects using either CD3+ or CD8+ as distinguishable TIL markers. Consequently, CD8+ intraepithelial TILs appear to be the gold standard for assessing her TIL prognosis in ovarian cancer. There was also a discrepancy between the presence of TILs and optimal surgical debulking. TILs have a more significant prognostic effect in Japanese and European patients than in North American patients, hypothesizing that immune modifiers such as genetic or environmental differences and access to medical care may influence survival. According to these independent studies, these TILs, especially CD8+ TILs, are good prognostic factors for ovarian cancer.[21]

CD8+ T lymphocytes plays a major role as cytotoxic killer cells. However, the ovarian cancer microenvironment harbours a vast network of immunosuppressive processes to stop attack by cytotoxic T cells, including recruitment of leukocytes with potent immunosuppressive activity. Tregs are important in maintaining self-tolerance by modulating immune responses to self-tissue antigens. However, numerous studies showed increased presence of Tregs in tumours that evade destruction by the immune system and that Tregs limit anti-tumour immunity. TGF and IL-10 cytokines and cell-cell interactions are used by regulatory Tregs to mediate inhibitory activity. Tregs immunosuppressive activity can be induced in ovarian cancer under hypoxic conditions within the tumour microenvironment.[27]

In some studies it was found that Treg cell recruitment to tumours was associated with decreased survival and increased risk of death. Tregs in the tumour environment and ascites are associated with poor prognosis of the patients. Tumours identified as TGF-secreting tumours are of advanced stage and grade. In ovarian cancer, it was observed in some studies that proper primary debulking is associated with decrease in Tregs and an increase in TILs response.[33]

Sato et al. [27] observed in a TIL subgroup with high CD8/CD4 ratio was observed to have a favourable survival prognosis, indicating an inhibitory role for Tregs. Fialova et al.[34] observed a shift in ovarian cancer patients with strong Th17 immune response in the early stages of cancer to the predominant her Treg population. They mentioned that it may be due to tumour progression leading to Treg involvement in the local immune environment.

In studies done on relationship between the stromal TIL with survival and prognosis it was observed that there was an association between the stromal TIL group with

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survival and prognosis. Hence it was concluded that TIL group can be considered as independent predictor for survival and prognosis.[2]

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Materials and methods:

Source of data: A prospective study was done on resected specimens of ovarian tumours sent to the histopathology section of the Department of Pathology, BLDE (Deemed to be University) Shri. B.M Patil Medical College Hospital & Research Centre, Vijayapura from January 2017–November 2022. (3 years of retrospective study & 2 years of prospective study).

Study period: December 2020–November 2022.

Study design: Descriptive (Cross-sectional study).

Methods of collection of data

Resected specimens of ovarian tumours received in the histopathology section of the Department of Pathology from January 2017 to November 2022, which were diagnosed as ovarian carcinoma in the histopathology section, were evaluated for subtyping and grading of the ovarian tumour. H&E stained sections of tumour tissues will be evaluated for TILs tumour tissue blocks on which TILs were evaluated and processed for IHC CD8+ T lymphocyte markers.

Method of counting tumour infiltrating lymphocytes:

TILs were evaluated as per a study done by Oktari et al.[5] Ten foci consisting of five intratumoral areas and five stromal areas with the highest TIL density were selected. Then on high power that is 400x, the number of lymphocytes in each focus were counted. Density analysis was done by adding up all the lymphocytes from each focus, and the mean was determined.

CD8+ T lymphocyte markers were evaluated as per a study done by Oktari et al.[5] Ten foci consisting of five intratumoral areas and five stromal areas with the highest TIL density were selected. Then on high power that is 400x, the number of CD8+ T lymphocyte positive cells in each focus were calculated. Density analysis will be done by adding up all CD8+ T positive lymphocytes from each focus, and the mean were determined. The mean of TILs and mean of CD8 + T lymphocytes correlated were done with histologic subtyping and grading of ovarian carcinoma.

Sample Size:

With an anticipated Mean ± SD of MC 5.338±3.04 [(5)], the study would require a sample size of 38 cases with a 95**%** level of confidence and precision of one. The formula used for the calculation of sample size is

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- $N=\frac{z^2 S^2}{d^2}$

  Where Z= Z statistic at α level of significance

  d^2= Absolute error

  P= Proportion rate

  q= 100-p

Inclusion criteria: Histologically diagnosed malignant cases of ovarian carcinoma were included.

Exclusion criteria: Benign and borderline epithelial tumours, germ cell tumours, sex cord-stromal, and metastatic ovarian tumours were excluded.

STATISTICAL ANALYSIS

- The data obtained were entered in a Microsoft Excel sheet, and statistical analysis was done using a statistical package for the social sciences (Verson 20).
- Results were presented as Mean (Median) ± SD and percentages.
- Results were presented in the form of tables and diagrammatic presentation.

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RESULTS

The study was done on resected specimens of ovarian tumours sent to the histopathology section of the Department of Pathology for a total period of 5 years, 3 years retrospective & 2 years prospective.

Maximum number of patients were between the age group of 41- 50 years amounting to 44.74% followed by 51-60 years and 31-40 years. The youngest patient in the present study was 26 years and the eldest patient was 65 years. The mean age of the study population was 45.94 ± 8.53 SD years.

Table 1: Age distribution of study population (n= 40)

Sr no	Age group (years)	No. of patients	Percentage (%)
1.	21-30	2	5%
2.	31-40	7	17%
3.	41-50	18	45%
4.	51-60	12	30%
5.	61-70	1	3%
Total		**40**	**100**

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Fig 1. Pie diagram: Age distribution of study population

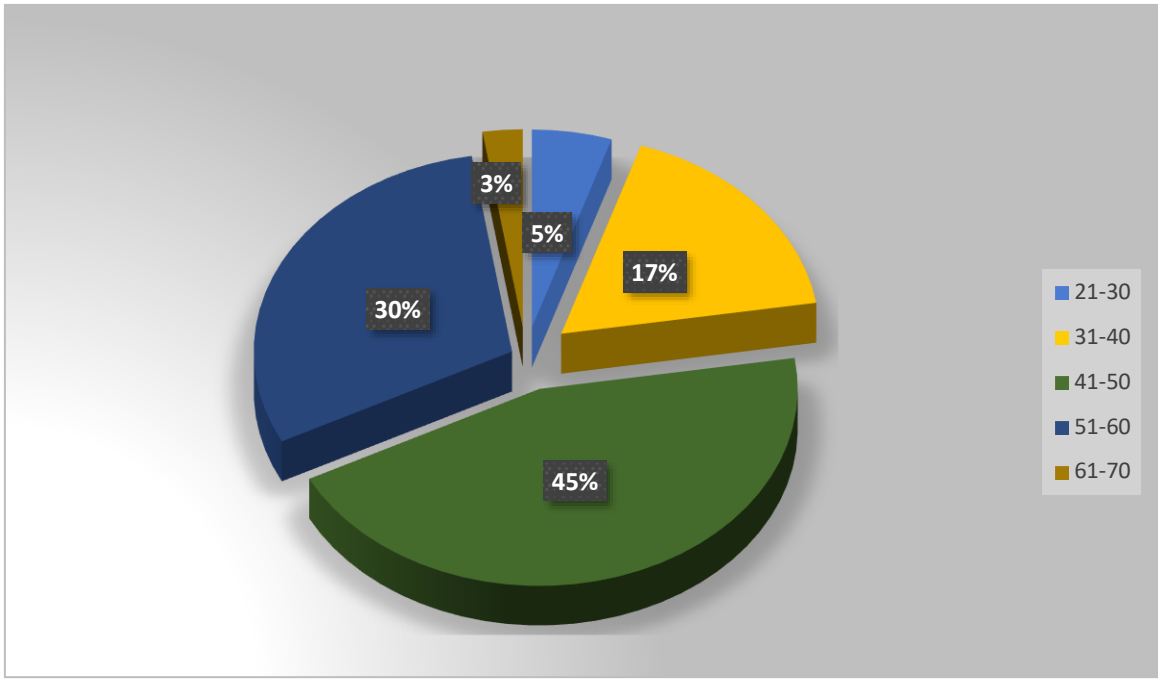

Table 2: Clinical presentation of study population (n=40)

Clinical presentation	**No of cases**	**Percentage (%)**
Abdominal pain	38	95 %
Mass per abdomen	30	75 %
Per vaginal bleeding	17	42.5 %
Ascites	3	7.5%
Pleural effusion	2	5%

Commonest clinical presentation in the present study was abdominal pain followed by mass per abdomen. In 7.5% cases ascites and in 5% cases pleural effusion was noted.

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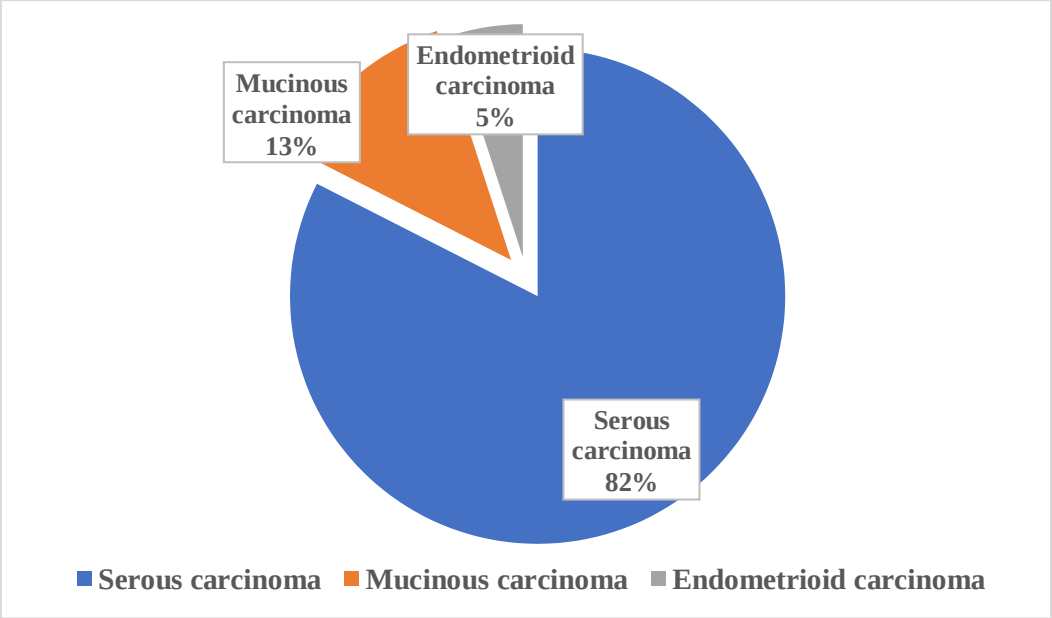
Table 3: Histopathological diagnosis of study population (n=40)

Sr no	Histopathological Subtypes	Number of cases	Grading	Percentage %
1.	Serous carcinoma	33	Low grade (n=18) High grade (n=15)	45% 37.5%
2.	Mucinous carcinoma	5	-	12.5%
3.	Endometrioid carcinoma	2	-	5%
	Total	**40**	**-**	**100%**

Fig 2. Pie diagram: Histopathological diagnosis of study population



Out of 40 patients evaluated 33 patients in the present study were diagnosed as serous carcinoma amounting to 82.5%. Out of the 33 serous carcinoma cases, 18 cases were classified as high grade serous carcinoma, whereas 15 cases were classified as low grade serous carcinoma. As per the WHO classification (2020) for mucinous and endometrioid carcinoma grading was not done.

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Table 4: Mean Tumor Infiltrating Lymphocytes on Histopathology (H&E sections) (n =40)

Sr no	Mean TILs Range	HP DIAGNOSIS			Total no of cases
		Endometrioid Carcinoma	Mucinous carcinoma	Serous carcinoma	
1.	3.01-4.00	0	0	5	5
2.	4.01-5.00	0	1	10	11
3.	5.01-6.00	2	1	8	11
4.	6.01-7.00	0	2	7	9
5.	7.01-8.00	0	0	3	3
6.	8.01-9.00	0	1	0	1
Total		**2**	**5**	**33**	**40**

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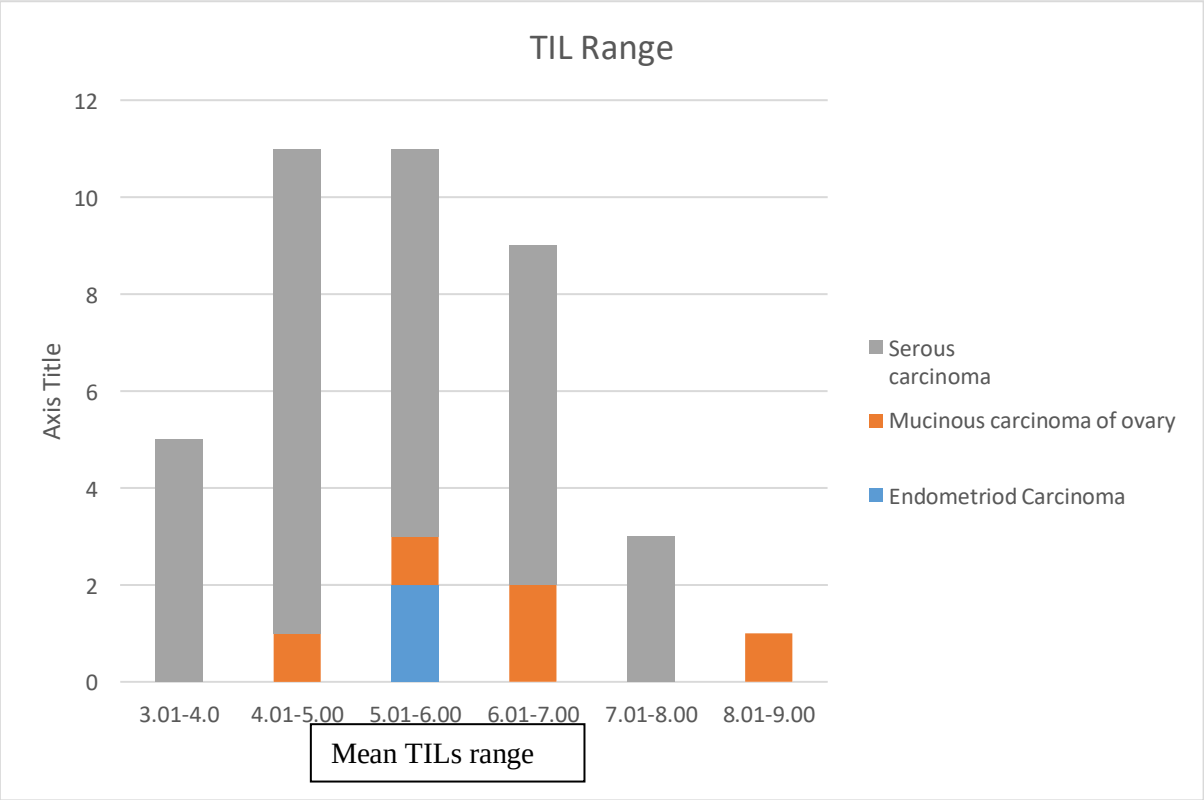
Fig 3. Bar diagram: Mean Tumor Infiltrating Lymphocytes on Histopathology



In 11 cases each mean TIL range was 4.01 -5.00 & 5.01 - 6.00. In 9 cases mean TIL range was 6.01- 7.00. In only 1 case highest mean TILs ranging from 8.01 – 9.00 was noted. Lowest mean TIL ranging from 3.01 – 4.00 was noted in 5 cases. The average mean ± SD of TILs in H&E sections was 5.46 ± 1.18.

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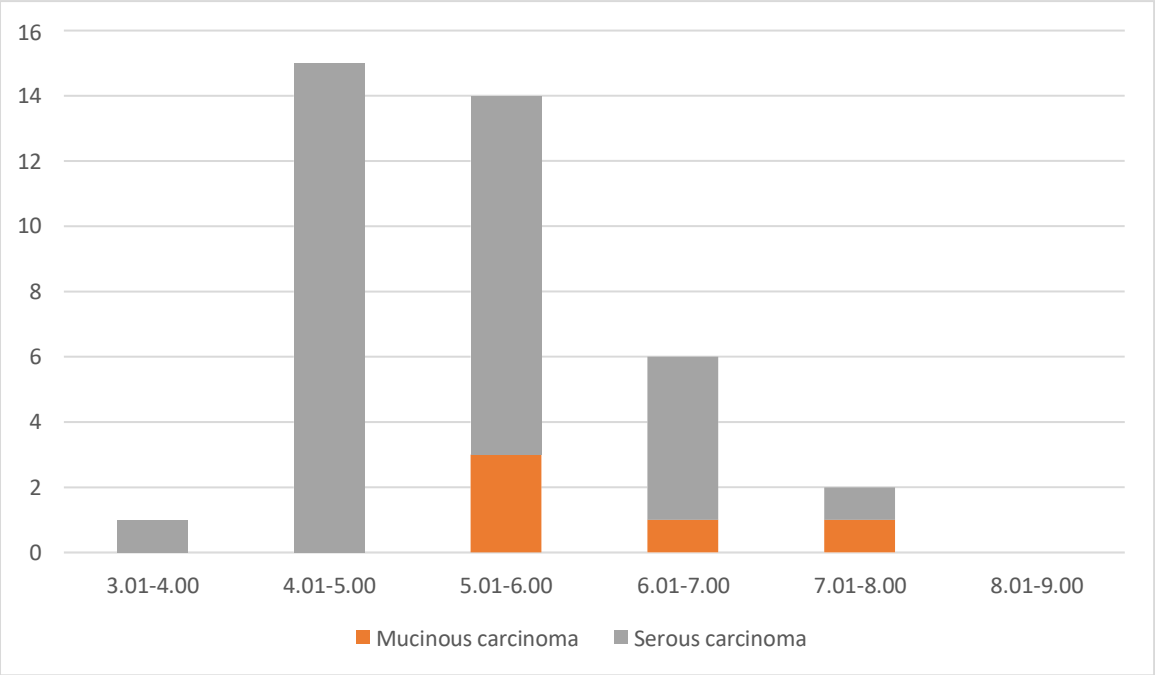
Table 5: Mean CD8+ T lymphocytes on Immunohistochemistry (n=38)

Sr no	Mean CD8+ TILs Range	Serous carcinoma	Mucinous carcinoma	Total no of cases
1.	3.01-4.00	1	0	1
2.	4.01-5.00	15	0	15
3.	5.01-6.00	11	3	14
4.	6.01-7.00	5	1	6
5.	7.01-8.00	1	1	2
6	8.01-9.00	0	0	0
Total		**33**	**5**	**38**

Fig 4. Bar diagram: Mean CD8+ lymphocytes on IHC (n=38)



On IHC in 15 cases, mean CD8+ TILs range was 4.01 - 5.00. In 14 cases CD8+ TIL range was 5.01 - 6.00. In 6 cases mean CD8+ TIL range was 6.01- 7.00. Highest mean CD8+ TILs range was 7.01 - 8.00, which was noted in 2 cases. There were no cases noted in which CD8+ TIL was ranging from 8.01 - 9.00. The average mean ± SD of mean CD8+ lymphocytes

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was 5.38 ± 0.85. Mean CD8+ lymphocytes on IHC was not done on endometrioid carcinoma due to non-availability of the blocks.

Table 6: Grading of serous carcinoma on study population (n=40)

Sr no	Grading	Number of cases	Percentage (%)
1.	High	18	45%
2.	Low	15	37.5%
3.	No Grading	7	17.5%
Total		**40**	**100.0**

Fig 5. Bar diagram: Grading of serous carcinoma on study population

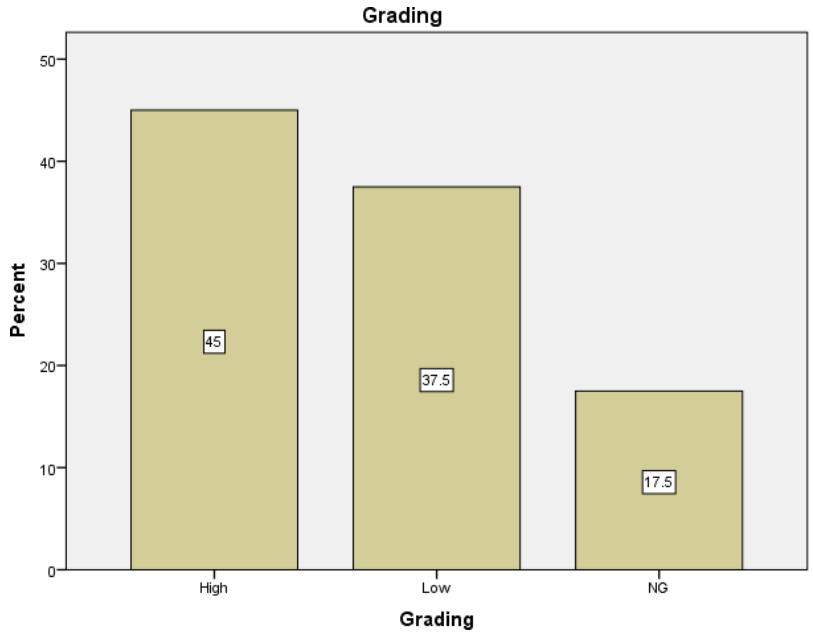

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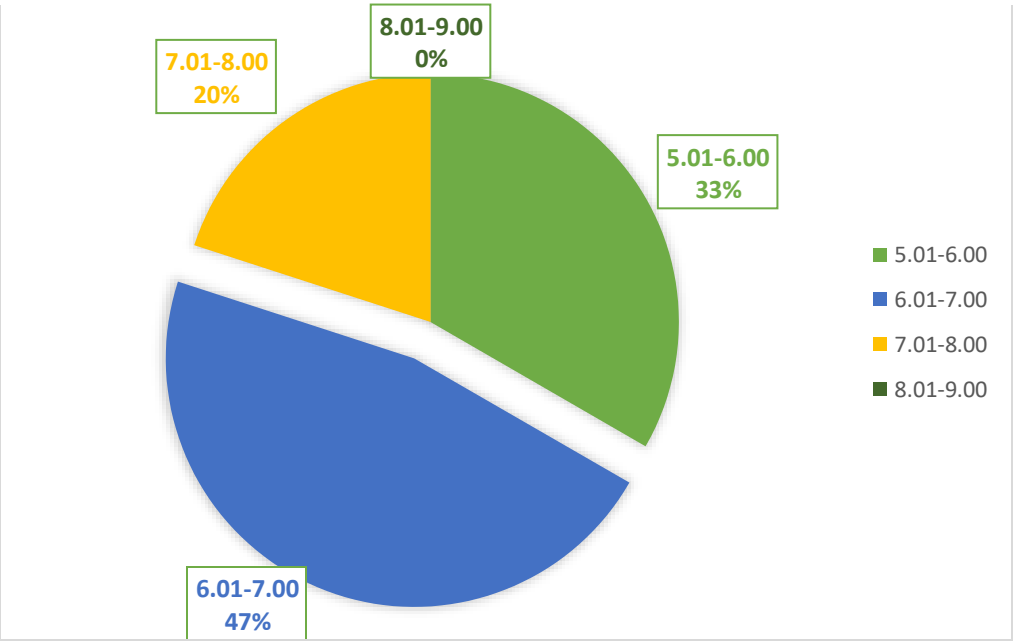
Table 7: Mean TILs in Low grade serous carcinoma (n=15)

Sr no	Mean TILs range	Number of cases	Percentage (%)
1	3.01 - 4.00	0	0%
2	4.01 - 5.00	0	0%
3	5.01 - 6.00	5	33.30%
4	6.01 - 7.00	7	46.70%
5	7.01 - 8.00	3	20.00%
6	8.01- 9.00	0	0%
Total		**15**	**100%**

Fig 6. Pie chart: Mean TILs in Low grade serous carcinoma



In this study, 15 patients were diagnosed with low grade serous carcinoma. In 7 cases mean TILs range was 6.01 - 7.00, 5 cases has mean TILs ranging from 5.01 - 6.00 and 3 cases has mean TILs ranging from 7.01 - 8.00. There were no cases noted in mean TILs ranging from 8.01 - 9.00. The average mean ± SD of mean TILs in H&E sections was 6.51 ± 0.72.

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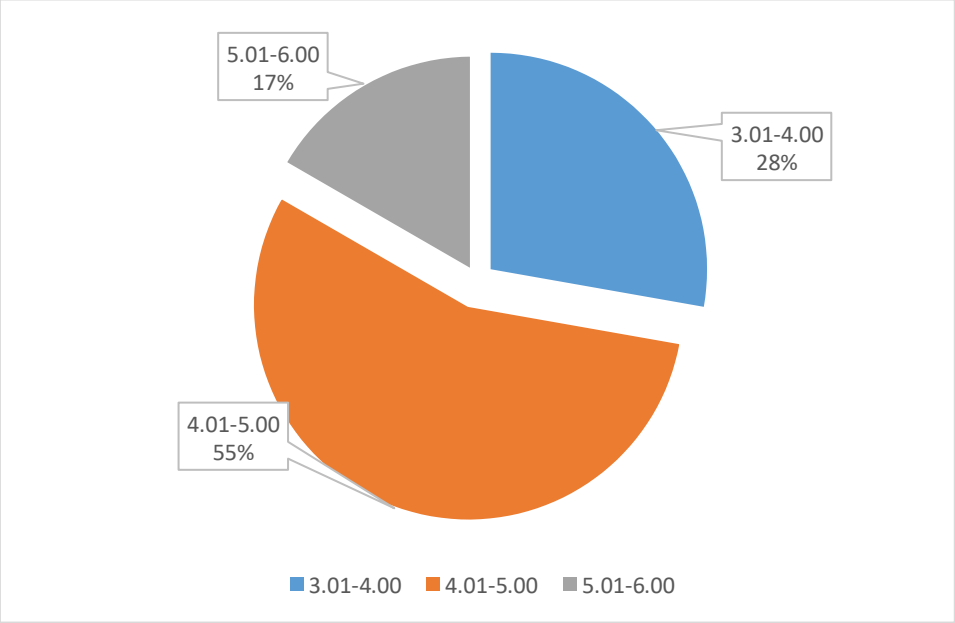
Table 8: Mean TILs in High grade serous carcinoma (n=18)

Sr no	Mean TILs range	Number of cases	Percentage (%)
1	3.01 - 4.00	5	27.80%
2	4.01 - 5.00	10	55.60%
3	5.01 - 6.00	3	16.70%
4	6.01 - 7.00	0	0%
5	7.01 - 8.00	0	0%
6	8.01 - 9.00	0	0%
Total		**18**	**100%**

Fig 7. Pie chart: Mean TILs in High grade serous carcinoma



Out of 18 patients diagnosed with high grade serous carcinoma, in 10 cases mean TILs range was 4.01 - 5.00. Five patients has mean TILs ranging from 3.01 – 4.00 and 3 patients has mean TILs ranging from 5.01 – 6.00. There were no cases noted in mean TILs ranging from

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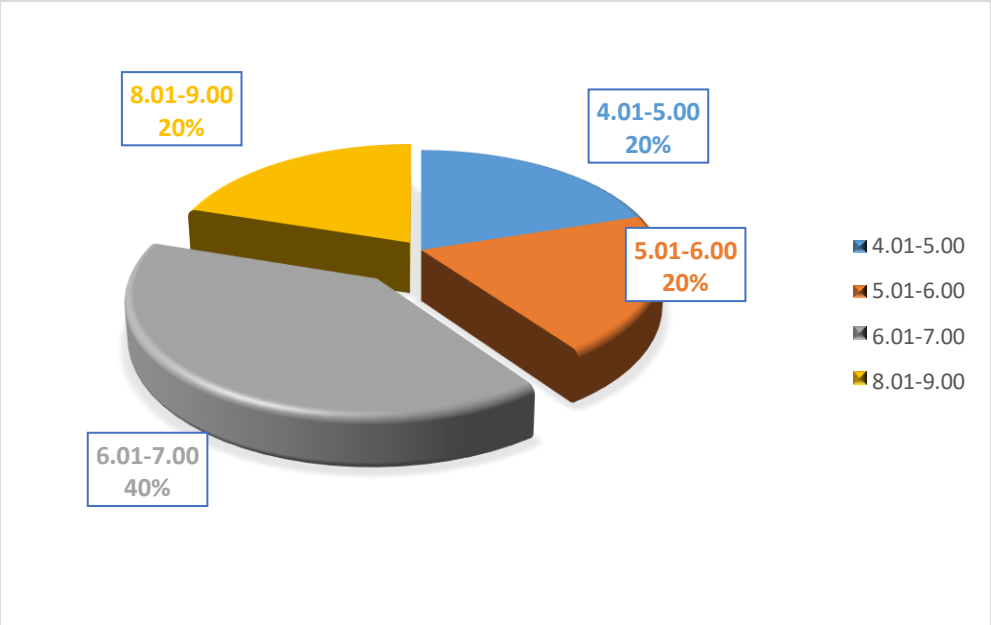
6.01 - 7.00, 7.01 - 8.00 and 8.01 - 9.00. The average mean ± SD of mean TILs in H&E sections was 4.52 ± 0.55.

Table 9: Mean TILs in Mucinous carcinoma (n=5)

Sr no	Mean TILs range	Number of cases	Percentage (%)
1	3.01 - 4.00	0	0%
2	4.01 - 5.00	1	20%
3	5.01 - 6.00	1	20%
4	6.01 - 7.00	2	40%
5	7.01 - 8.00	0	0%
6	8.01 - 9.00	1	20%
Total		**5**	**100%**

Fig 8. Pie chart: Mean TILs in mucinous carcinoma



Five patients were diagnosed with mucinous carcinoma. In 2 cases mean TILs range was 6.01 - 7.00. On H&E 1 case each, has mean TILs ranging from 4.01 - 5.00, 5.01 - 6.00 and 8.01 - 9.00 respectively. There were no cases noted in mean TILs ranging from 7.01 - 8.00. The average mean ± SD of mean TILs in H&E sections was 6.36 ± 1.24.

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Table 10: Mean TILs in Endometrioid carcinoma (n=2)

Sr no	Mean TILs	Number of cases	Percentage (%)
1	3.01 – 4.00	0	0%
2	4.01 - 5.00	0	0%
3	5.01 - 6.00	1	50%
4	6.01 - 7.00	1	50%
5	7.01 - 8.00	0	**0%**
6	8.01 - 9.00	0	**0%**
Total		**2**	**100%**

Two patients were diagnosed with endometrioid carcinoma, each case has mean TILs ranging from 5.01 – 6.00 and 6.01 – 7.00 respectively. The average mean ± SD of mean TILs in H&E sections was 5.7 ± 0.14.

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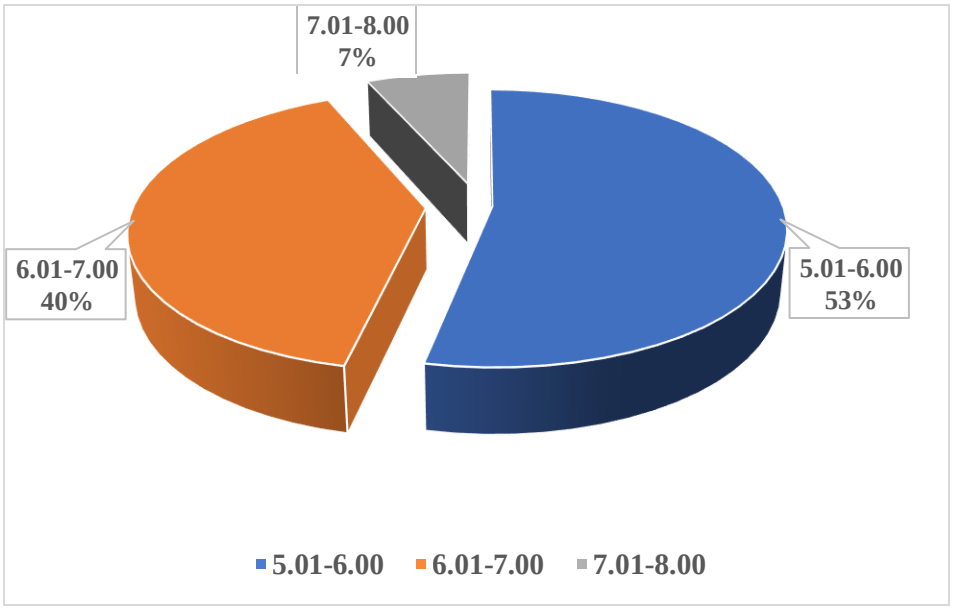
Table 11: Mean CD8+ lymphocytes in Low grade serous carcinoma (n=15)

Sr no	Mean CD8+ TILs range	Number of cases	Percentage (%)
1	3.01 – 4.00	0	0%
2	4.01 - 5.00	0	0%
3	5.01 - 6.00	8	53.30%
4	6.01 - 7.00	6	40.00%
5	7.01 - 8.00	1	6.70%
6	8.01 - 9.00	0	0%
Total		**15**	**100%**

Fig 9. Pie chart: Mean CD8+ lymphocytes in Low grade serous carcinoma



Out of 15 patients diagnosed with low grade serous carcinoma, 8 cases has mean CD8+ lymphocytes ranging from 5.01 – 6.00. Six cases has mean CD8+ lymphocytes ranging from 6.01 - 7.00 and only 1 case has mean CD8+ lymphocytes ranging from 7.01 – 8.00. There were no cases noted in mean CD8+ TILs ranging from 4.01 - 5.00 and 8.01 - 9.00. The average mean ± SD of mean CD8+ lymphocytes was 5.98 ± 0.67.

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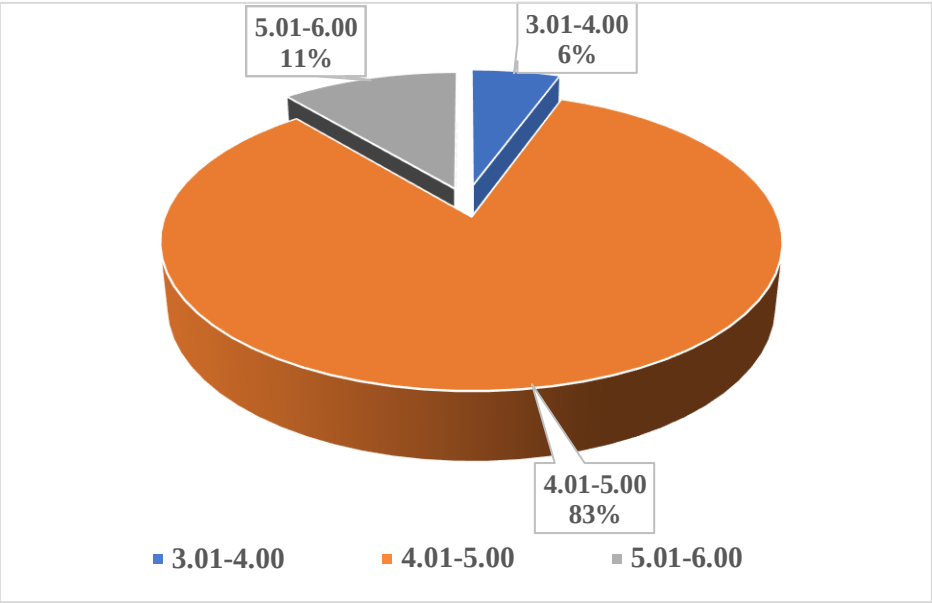
Table 12: Mean CD8+ lymphocytes in High grade serous carcinoma (n=18)

Sr no	Mean CD8+ TILs range	Number of cases	Percentage (%)
1	3.01 - 4.00	1	5.60%
2	4.01 - 5.00	15	83.30%
3	5.01 - 6.00	2	11.10%
4	6.01 – 7.00	0	0%
5	7.01 – 8.00	0	0%
6	8.01 – 9.00	0	0%
Total		**18**	**100%**

Fig 10. Pie chart: Mean CD8+ lymphocytes in High grade serous carcinoma



Out of 18 patients diagnosed with high grade serous carcinoma, 15 patients has mean CD8+ lymphocytes ranging from 4.01 – 5.00. Two patients has mean CD8+ lymphocytes ranging from 5.01 – 6.00 and 1 case has mean CD8+ lymphocytes ranging from 3.01 – 4.00. There were no cases noted in mean CD8+ TILs ranging from 6.01 - 7.00, 7.01 – 8.00 and 8.01 - 9.00. The average mean ± SD of mean CD8+ lymphocytes was 4.7 ± 0.45.

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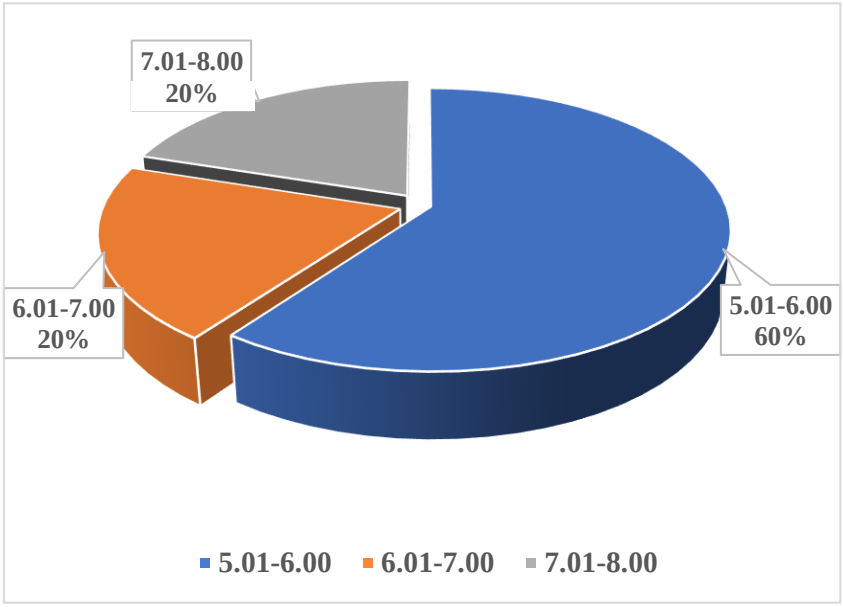
Table 13: Mean CD8+ lymphocytes in Mucinous carcinoma (n=5)

Sr no	Mean CD8+ TILs range	Number of cases	Percentage (%)
1	3.01 - 4.00	0	0%
2	4.01 - 5.00	0	0%
3	5.01 - 6.00	3	60%
4	6.01 - 7.00	1	20%
5	7.01 - 8.00	1	20%
6	8.01 - 9.00	0	0%
Total		**5**	**100%**

Fig 11. Pie chart: Mean CD8+ lymphocytes in Mucinous carcinoma



Out of 5 patients diagnosed with mucinous carcinoma, 3 cases has mean CD8+ TILs ranging from 5.01 – 6.00. One case each has mean CD8+ TILs ranging from 6.01 - 7.00 and 7.01 - 8.00 respectively. There were no cases noted in mean CD8+ TILs ranging from 3.01 - 4.00, 4.01 – 5.00 and 8.01 - 9.00. The average mean ± SD of mean CD8+ TILs was 6.15 ± 0.89.

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Statistical analysis of non-parametric variables between mean TILs and mean CD8+ TILs groups were compared using the Spearman's rho test. The coefficient of correlation value between mean TILs and mean CD8+ TILs, which is statistically significant with r=0.756.
Mean TILs and mean CD8+ TILs are correlating significantly at the 0.01 level (2-tailed). P-value = 0.00 was considered statistically significant. All statistical tests were performed two-tailed.

Variable	**Spearman's rho correlation coefficient value (r)**	**p-value**
Mean TILs v/s CD8+ TILs	0.756**	0.000

**. Correlation is significant at the 0.01 level (2-tailed).

CORRELATION BETWEEN MEAN TILs AND CD8+ TILs

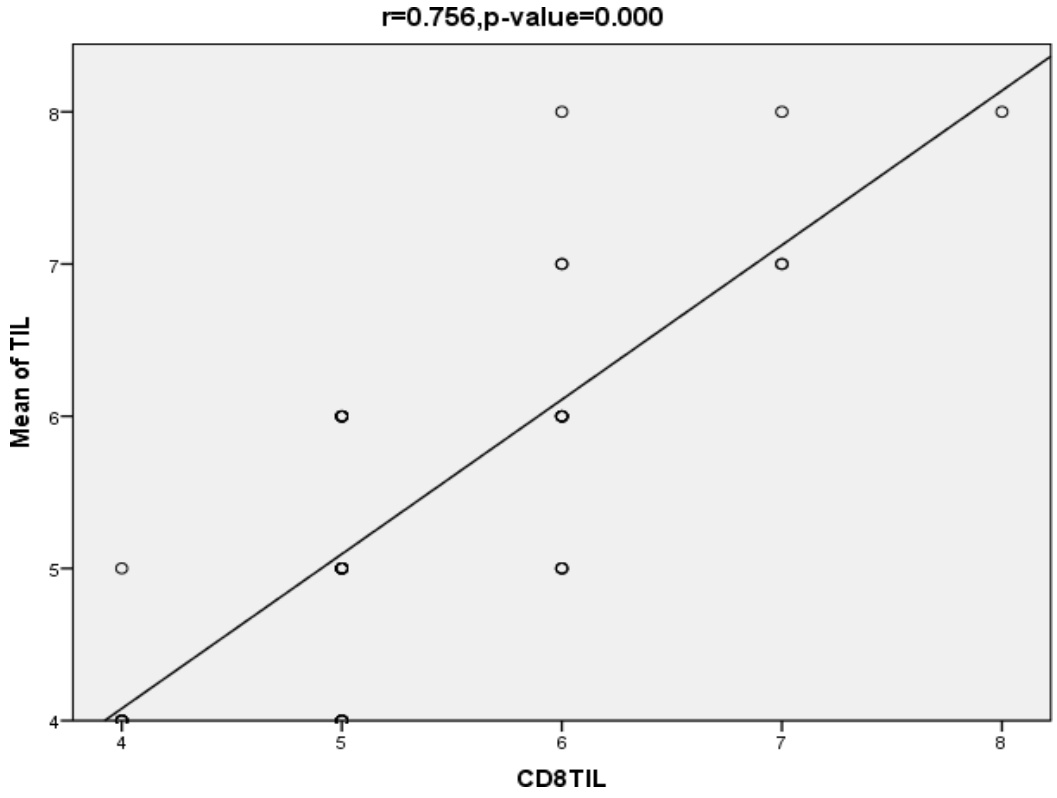

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TABLE 14: ASSOCIATION OF MEAN TILs AND CD8+ TILs DENSITY WITH HISTOPATHOLOGICAL SUBTYPES

Histopathological Subtype		Mean	Std. Deviation	Std. Error	Minimum	Maximum	P*value
Mean TILs	Endometrioid carcinoma	5	0	0	5	5	0.370
	Mucinous carcinoma	6.2	1.304	0.583	5	8	
	Serous carcinoma	5.39	1.197	0.208	4	8	
CD8+ TILs	Endometrioid carcinoma	-	-	-	-	-	0.035
	Mucinous carcinoma	6.4	1.14	0.51	5	8	
	Serous carcinoma	5.18	0.808	0.141	4	7	

***Kruskal Wallis, significant if p<0.05**

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TABLE 15: COMPARISON OF MEAN TILs AND CD8+ TILs DENSITY WITH LOW AND HIGH GRADE OF SEROUS CARCINOMA

Histopathological grading of serous carcinoma		Mean	Std. Deviation	Std. Error	Minimum	Maximum	P*value
Mean TILs range	Low grade serous carcinoma	6.3	0.67	0.175	5.5	7.6	< .001
	High grade serous carcinoma	4.5	0.54	0.128	3.6	5.6	
CD8+ TILs	Low grade serous carcinoma	5.9	0.55	0.142	5.2	7.0	< .001
	High grade serous carcinoma	4.6	0.38	0.106	3.9	5.4	

Note: Student's t- Test * p < .001

Based on results of independent samples t-test, there was no statistically significance difference between the mean TILs & CD8+ T lymphocytes and grading of serous carcinoma ($p < .001$).

When comparison of mean TILs and CD8+ T lymphocytes with low and high grade of serous carcinoma was done. It was observed that mean TILs density and CD8+ TILs expression was higher in low grade serous carcinoma as compared to high grade.

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PHOTOMICROGRAPHS

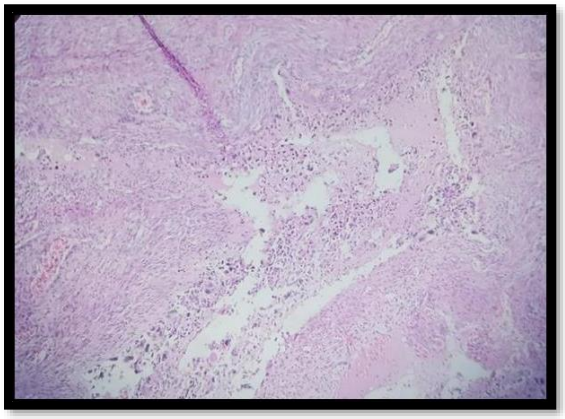

Fig 13- Photomicrograph of TILs in low grade serous carcinoma (H&E, 100X)

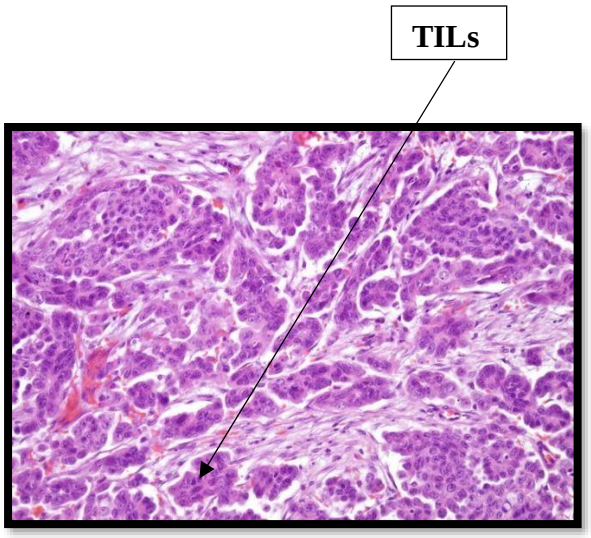

Fig 14- Photomicrograph of TILs in low grade serous carcinoma (H&E, 400X)

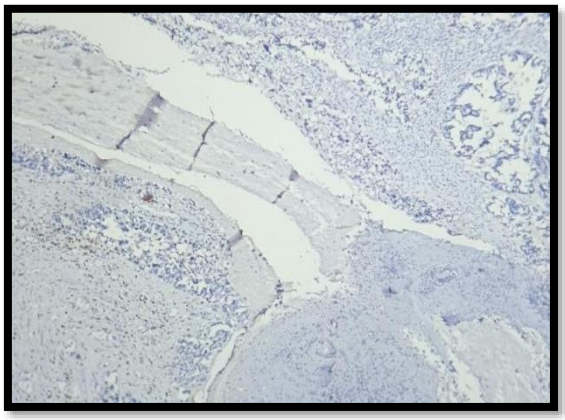

Fig 15 - Photomicrograph of CD8+ T lymphocytes in low grade serous carcinoma (CD8+, 100X)

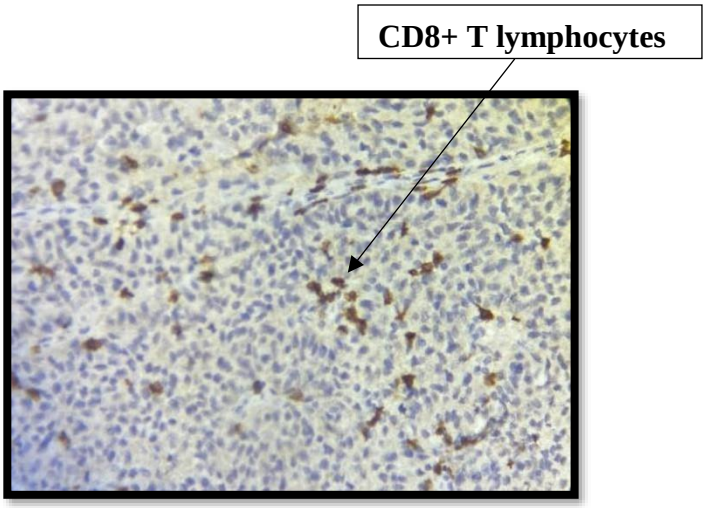

Fig 16 - Photomicrograph of CD8+ T lymphocytes in low grade serous carcinoma (CD8+, 400X)

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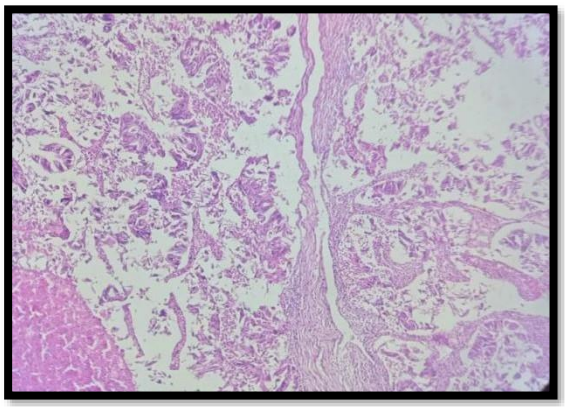

Fig 17- Photomicrograph of TILs in high grade serous carcinoma (H&E, 100X)

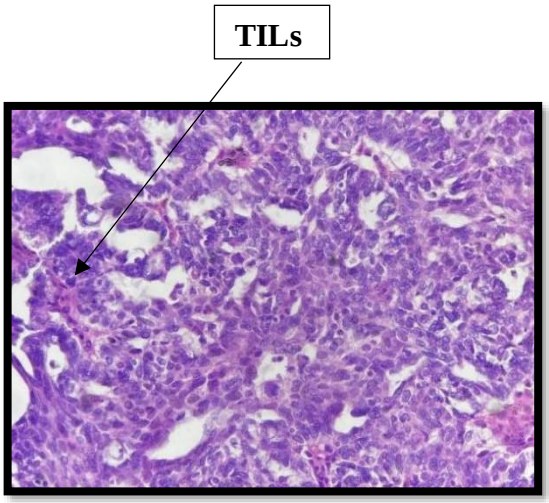

Fig 18- Photomicrograph of TILs in high grade serous carcinoma (H&E, 400X)

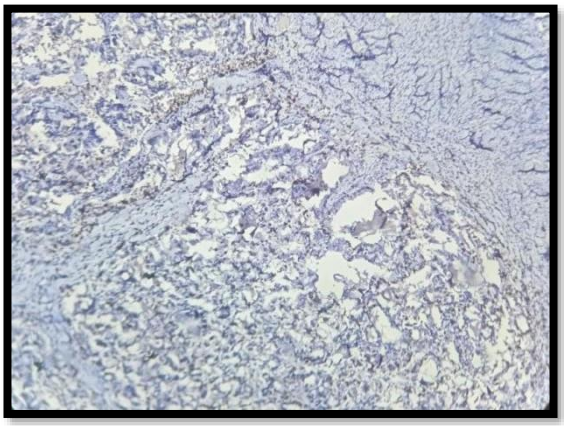

Fig 19 - Photomicrograph of CD8+ T lymphocytes in high grade serous carcinoma (CD8+, 100X)

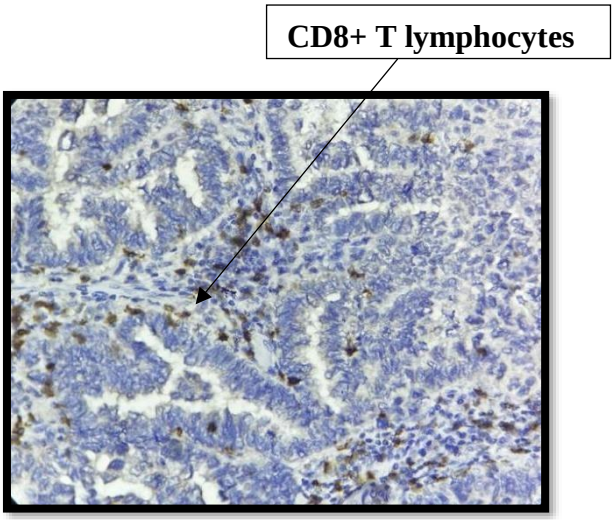

Fig 20 - Photomicrograph of CD8+ T lymphocytes in high grade serous carcinoma (CD8+, 400X)

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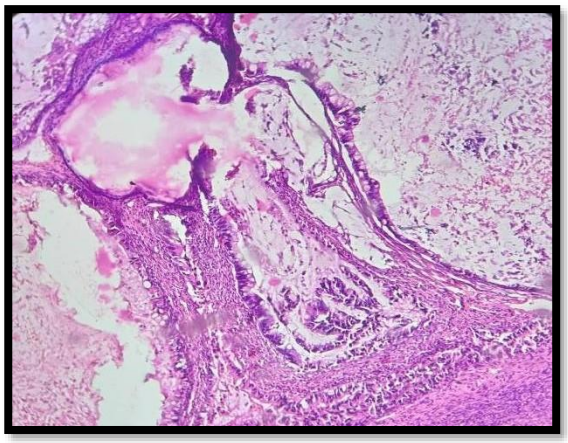

Fig 21 - Photomicrograph of TILs in mucinous carcinoma (H&E, 100X)

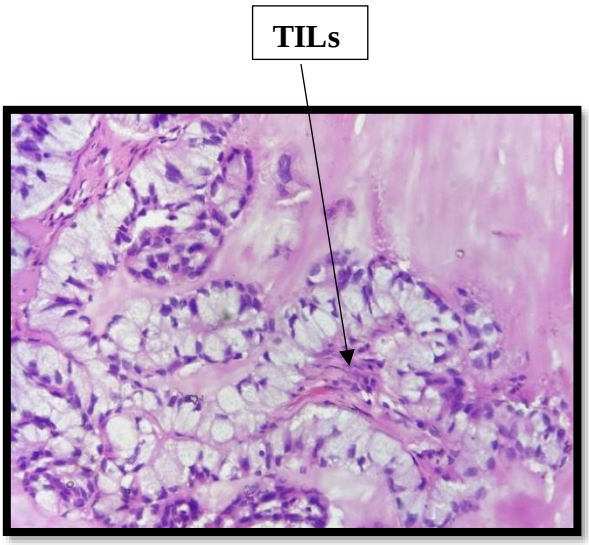

Fig 22 - Photomicrograph of TILs in mucinous carcinoma (H&E, 400X)

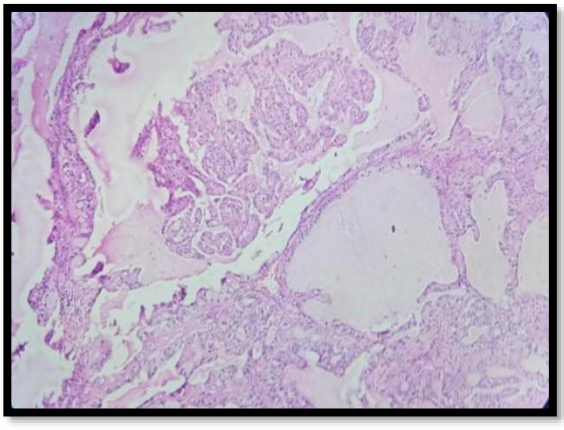

Fig 23 - Photomicrograph of TILs in mucinous carcinoma (H&E, 100X)

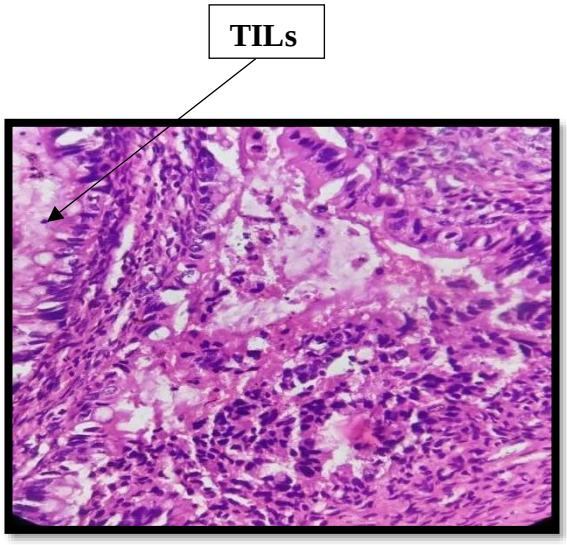

Fig 24 - Photomicrograph of TILs in mucinous carcinoma (H&E, 400X)

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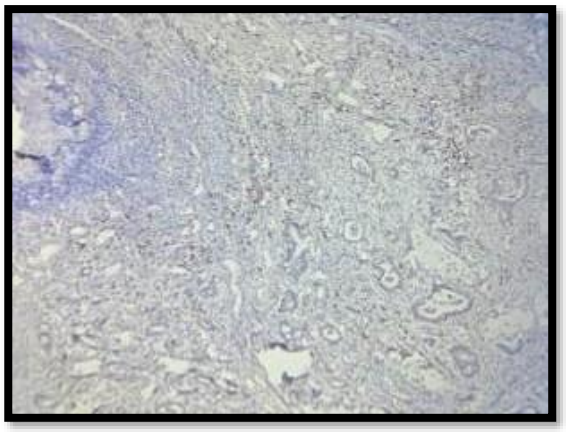

Fig 25 - Photomicrograph of CD8+ T lymphocytes in mucinous carcinoma (CD8+, 100X)

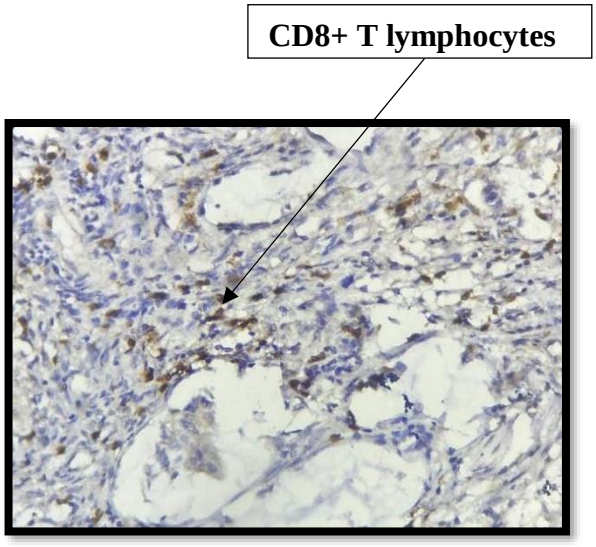

Fig 26 - Photomicrograph of CD8+ T lymphocytes in mucinous carcinoma (CD8+, 400X)

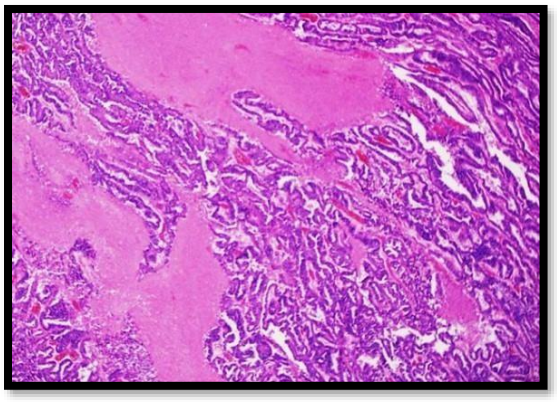

Fig 27 - Photomicrograph of TILs in endometrioid carcinoma (H&E, 100X)

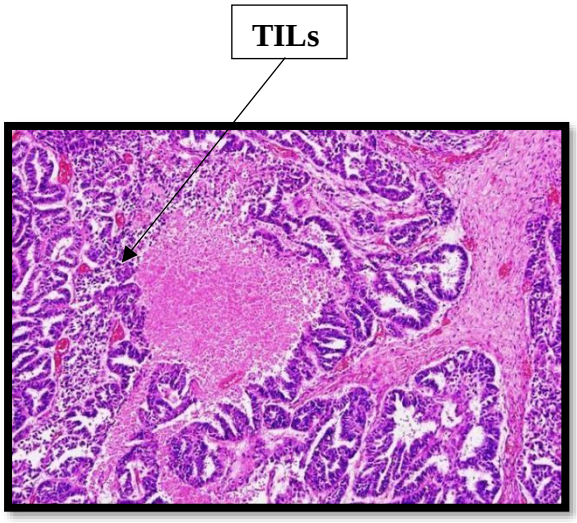

Fig 28 - Photomicrograph of TILs in endometrioid carcinoma (H&E, 400X)

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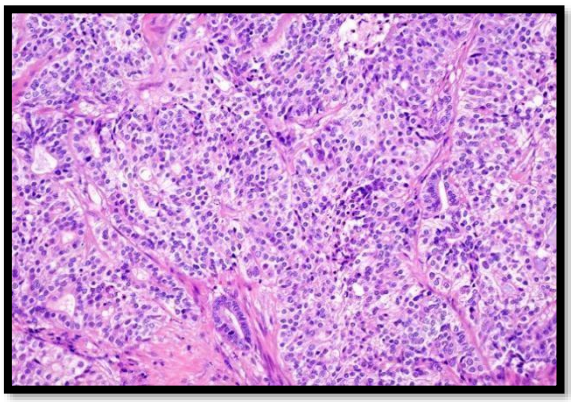

Fig 29 - Photomicrograph of TILs in endometrioid carcinoma of case 2 (H&E, 100X)

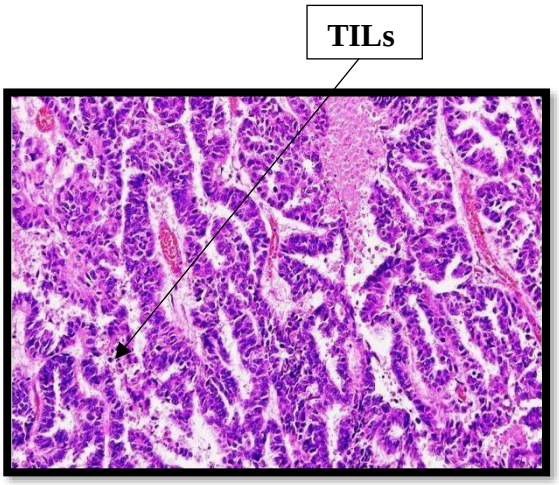

Fig 30 - Photomicrograph of TILs in endometrioid carcinoma of case 2 (H&E, 400X)

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DISCUSSION

In the present study, total 40 cases of malignant ovarian cancers were analysed. Mean of TILs and mean of CD8+ T Lymphocytes with high TIL density was done. Association of tumour infiltrating lymphocytes (TILs) with histopathological subtyping and grading of serous carcinoma was evaluated. In mucinous and endometrioid carcinoma association of grading with TIL and CD8+ T lymphocytes was not done as in 2020 WHO classification, grading was not suggested for these subtypes

In the present study mean age range for ovarian carcinoma was 26 to 65 years similar findings were noted in the study done by Desyani et al.[5] having age range of 21 to 71 years. In other studies age range for ovarian carcinoma was from 31 to 83 years.[3, 32, 34]

Oktari et al.[5] in their study mentioned that old age is a risk factor for malignancy, it can be due to onset of somatic mutation. In the present study also maximum number of cases were noted between the age group of 41- 50 years. This may be due to accumulation of somatic mutations in elderly age group as mentioned in the study done by Oktari et al.[5]

Out of 40 patients in this study, maximum number of cases were clinically presented with pain in abdomen amounting to 95% followed by irregular vaginal bleeding amounting to 42.5%. These findings are similar to study done by Krishnaswamy P et al. [35]

In the study done by Hwang et al [28] on clinical significance of TILs in ovarian cancer, maximum number of cases were diagnosed as serous carcinoma of ovary amounting to 56% followed by mucinous carcinoma amounting to 19 % and endometrioid carcinoma of ovary was the least diagnosed ovarian carcinoma. On histopathological study of ovarian carcinoma maximum number of cases were diagnosed as serous carcinoma of ovary amounting to 82.5% followed by mucinous carcinoma amounting to 12.5% and endometrioid carcinoma of ovary was the least diagnosed ovarian carcinoma. These findings are similar to study done by Krishnaswamy P et al. [35] and Hwang et al [28].

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Table 16: Histopathological subtypes of ovarian tumours in various studies and present study

	Serous carcinoma (%)	**Mucinous carcinoma (%)**	**Endometrioid carcinoma (%)**
Hwang et al [28]	56	19	8
Krishnaswamy P et al. [35]	43	14	10
Jun li et al. [36]	61	16	7
Peres et al. [3]	69.5	14.5	8
Present study	82.5	12.5	5

In the study done by Oktari et al.[5] on whether TILs can help to identify CD8+ tumour infiltrating lymphocytes and histopathological subtypes of ovarian carcinoma, observed that there was a significant correlation between intratumoral TILs density and the histopathologic subtypes of ovarian cancer having p value of 0.020 based on the results of Kruskal-Wallis test. There was no correlation observed between intrastromal TILs and histopathological subtypes of ovarian cancer in their study. However in their study TIL density was significantly associated with intratumoral and stromal CD8+ TILs ($p < 0.05$).

Sato E et al.[32] evaluated TILs in epithelial ovarian carcinoma using IHC marker CD8+TILs. They observed that cases with more TILs density of CD8+ T cells survived longer when compared to low TILs density cases ($P = 0.0003$). They concluded that intraepithelial CD8+ TILs are associated with better prognosis in epithelial ovarian carcinoma.

In present study, mean ± SD of TILs in H&E sections was 5.46 ± 1.18 whereas mean ± SD of mean CD8+ lymphocytes was 5.38 ± 0.85. On evaluation of mean TILs and mean CD8+ TILs on histopathologic subtypes of ovarian carcinoma based on spearman's rho test results it was observed that coefficient of correlation value is statistically significant with r=0.756. Mean TILs and mean CD8+ TILs are correlating significantly at the 0.01 level (2-

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tailed). There was also significant association found between CD8+ TILs and histopathologic subtypes of ovarian carcinoma based on Kruskal-Wallis test (p=0.035).

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SUMMARY

- This study was done on resected specimens of ovary sent to the histopathology section of the Department of Pathology, BLDE (Deemed to be University) Shri. B.M Patil Medical College Hospital & Research Centre, Vijayapura for a total period from January 2017–November 2022 (3 years retrospective & 2 years prospective).
- The present study included 40 cases of malignant ovarian cancers as per the inclusion and exclusion criteria. Maximum number of patients were between the age group of 41- 50 years amounting to 45%. The youngest patient in the present study was 26 years and the eldest patient was 65 years. The mean age of the study population was 45.94 ± 8.53 SD years.
- Out of 40 patients evaluated 33 patients in the present study were diagnosed as serous carcinoma amounting to 82.5%. 5 patients were diagnosed as mucinous carcinoma. 2 patients were diagnosed as endometrioid carcinoma. Out of the 33 serous carcinoma cases, 18 cases was classified as high grade serous carcinoma, whereas 15 cases was classified as low grade serous carcinoma.
- Maximum number of study cases amounting to 22 has mean TILs ranging from 4.01 - 5.00 & 5.01 - 6.00 and only 1 case has mean TILs ranging from 8.01 – 9.00. The average mean ± SD of mean TILs is 5.46 ± 1.18.
- Maximum number of cases diagnosed with serous carcinoma amounting to 76% has mean CD8+ lymphocytes ranging from 4.01 - 5.00 & 5.01 - 6.00 and only 1 patient has mean CD8+ lymphocytes ranging from 3.01 – 4.00. The average mean ± SD of mean CD8+ lymphocytes is 5.38 ± 0.85.
- When comparison of TIL and CD8+ T lymphocytic infiltrate between low and high grade serous carcinoma was done, it was observed that TIL infiltrate and CD8+ T lymphocyte expression was higher in low grade serous carcinoma when compared to high grade serous carcinoma.
- The Mean of CD8+ T Lymphocytes count is significantly associated with grading of serous carcinoma of ovary. Hence CD8+ TILs can be used for predictive factor for prognosis.

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CONCLUSION

In the present study, TILs density in both H&E sections and CD8+ TILs showed a significant correlation with degree of the association. This shows that higher the number of TILs, higher the density of CD8+ TILs expression. This study shows association between the mean of CD8 + T lymphocytes with histopathological subtyping and grading of ovarian carcinoma. These findings in our study suggest that TILs can be used as predictive biomarkers for the prognosis of ovarian cancer patients. However multicentric meta-analysis of TILs in ovarian carcinoma with CD8+ T lymphocyte markers may help to conclude TILs and CD8+ T lymphocytes as a prognostic biomarkers in ovarian carcinoma.

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ANNEXURE-I

ETHICAL CLEARANCE

B.L.D.E. (DEEMED TO BE UNIVERSITY) IEC/No-9/2021 Date-22/01/2021

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Evaluation of tumour lymphocytic infiltration in ovarian carcinoma and its correlation with various subtypes and grading of ovarian carcinoma

Name of PG student: Dr Harish Kumar K, Department of Pathology

Name of Guide/Co-investigator: Dr S U Arakeri , Professor & HOD of Pathology

DR .S.V.PATIL
CHAIRMAN, IEC
Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

2

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ANNEXURE- II

B.L.D.E. (DEEMED TO BE UNIVERSITY)

SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA.

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned,_____, S/O D/O W/O_____, aged_____years, ordinarily resident of_____do hereby state/declare that Dr_____of _____ Hospital has examined me thoroughly on _____ at _____(place) and it has been explained to me in my own dialect that I am suffering from_____disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled _____under the guidance of Dr_____requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

The Doctor has also informed me that during the conduct of this procedure, adverse results may be encountered. Among the above complications, most of them are treatable but are not anticipated; hence there is a chance of aggravation of my condition, and in rare circumstances, it may prove fatal despite the anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in the evaluation of the results of the study, which is a useful reference to the treatment of other similar cases in the near future. Also, I may be benefited in getting relieved from suffering or cure of the disease I am suffering.

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The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than my legal hirer or me except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on the information given by me, I can ask for any clarification during treatment/study related to diagnosis, the procedure of treatment, result of treatment or prognosis. In the meantime, I have been informed that I can withdraw from my participation in this study at any time if I want, or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt_____under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of the patient:

Signature of the Doctor:

Witness: 1.

2.

Date:

Place

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ANNEXURE- III

PROFORMA FOR STUDY:

NAME : OP/IP No. :

AGE :

OCCUPATION :

RESIDENCE :

Presenting Complaints :

Past history :

Personal history :

Family history :

Treatment history : History of corticosteroids and any other drugs

General physical examination:

Pallor present/absent

Icterus present/absent

Clubbing present/absent

Lymphadenopathy present/absent

Built poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE:

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SYSTEMIC EXAMINATION:

CLINICAL DIAGNOSIS:

TREATMENT:

INVESTIGATIONS:

1. Histopathological study

i) Histopathological subtyping

ii) Grading of the tumour

iii) TIL count

2. Immunohistochemistry for CD8+ T Lymphocytes

Table 1: Evaluation of cases of ovarian carcinoma- Association between the mean of TILs and the mean of CD8 + T lymphocyte infiltration with histological subtyping and grading of ovarian carcinoma

Sr.no	**Age**	**Histological subtype**	**Histological grading**	**Histopathological Diagnosis**	**Mean of TILs on H&E stained section**	**Mean of CD8+ T Lymphocytes count**

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MASTER CHART

S.NO	AGE	PATIENT NAME	HPR NO	OPD/IPD NO	GROSS	GRADING	HP DIAGNOSIS	Mean of TILs	IHC MARKER	Mean of CD8+
1	36	Geetha Kumbar	2483/21	IP-85406	Cystic, irregular, bossilated, focal areas of solid areas noted.	High	Serous carcinoma	3.89	CD8+	4.1
2	35	Bhagyashree	2979/21	IP-38824	Cystic, irregular, bossilated, focal areas of solid areas noted.	High	Serous carcinoma	5.12	CD8+	5.42
3	27	Sahera banu	2836/17	IP-147339	Cystic, focal solid areas noted	-	Mucinous carcinoma	8.16	CD8+	7.8
4	54	Abiba	2904/17	IP-14034	Cystic, focal solid areas with hemorrhage	Low	Serous carcinoma	5.6	CD8+	5.4
5	50	Meenakshi	287/20	IP-1318	Cystic, glistening, pale white	High	Serous carcinoma	4.74	CD8+	5
6	54	Channamma	5691/18	IP-23179	Cystic, glistening, pale white	High	Serous carcinoma	4.1	CD8+	4.55
7	36	Subhadra bai	4917/17	IP-23719	Cystic, focal solid areas noted	-	Mucinous carcinoma	6.56	CD8+	6.7
8	37	Aishwarya biswal	5674/18	IP-1895	Cystic, pale white	High	Serous carcinoma	4.37	CD8+	4.6
9	45	Alaka	1979/21	IP-21947	Cystic, glistening, pale white	High	Serous carcinoma	4.9	CD8+	5.26
10	51	Ammavva	6184/18	IP-351670	Cystic, pale white	High	Serous carcinoma	3.8	CD8+	4.28
11	55	Basawwa Siddappa	1466/21	IP-147495	Cystic, Multi nodular whitish areas noted. Drained 100ml of sticky material.	-	Mucinous carcinoma	5.4	CD8+	5.6
12	45	Jayashree Biradar	148/19	IP-2777	Cystic, Multi nodular whitish areas noted. Drained 10ml of sticky semi solid material.	-	Mucinous carcinoma	6.15	CD8+	5.75

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13	65	Shivamma	4421/19	OP-120786	Bossilated, solid, homogenous, pale yellow, friable areas noted.	Low	Serous carcinoma	5.8	CD8+	5.4
14	55	Gangamma	4878/19	IP-240024	Cystic, pale white	Low	Serous carcinoma	6.28	CD8+	5.86
15	60	Sugarbi	775/19	IP-34213	Cystic, glistening, pale white	High	Serous carcinoma	4.5	CD8+	4.69
16	50	Meenaxi Kumbar	1487/18	IP-1318	Cystic, glistening, pale white	High	Serous carcinoma	4.6	CD8+	4.55
17	43	Shabana	1959/20	IP-119912	Pale yellow, friable, papillary, nodular growth	Low	Serous carcinoma	7.68	CD8+	7.05
18	46	Kallasar	4646/17	OP-4009	Cystic, pale white	Low	Serous carcinoma	5.67	CD8+	5.12
19	48	Shobha hadari	1759/20	OP-679	Cystic, Multi nodular whitish areas noted. Drained 1000ml of sticky semi solid material.	Low	Serous carcinoma	7.1	CD8+	6.5
20	26	Savithri rathod	1108/17	IP-127908	Cystic, pale white	Low	Serous carcinoma	6.44	CD8+	6.3
21	42	Anjali	6893/17	IP-35427	Solid, hemorrhagic. Tan white on cut surface	High	Serous carcinoma	3.91	CD8+	4.56
22	42	Suhasini pawar	2458/19	IP-114177	Cystic, Drained 10ml of sticky colorless material.	-	Mucinous carcinoma	4.8	CD8+	5.28
23	53	Nasmeen Banu	698/18	IP-11805	Cystic, glistening, pale white	High	Serous carcinoma	4.6	CD8+	3.9
24	45	Indira chavan	1723/18	IP-11903	Cystic, pale white	High	Serous carcinoma	4.35	CD8+	4.77
25	46	Sharda patil	2101/18	IP-12049	Cystic, glistening, pale white	High	Serous carcinoma	3.6	CD8+	4.26
26	55	Jayalaxmi pujari	3768/20	IP-12254	Cystic, pale white	High	Serous carcinoma	5.6	CD8+	4.7
27	55	Gourabai gada	1554/19	IP-12521	Cystic, glistening, pale white	Low	Serous carcinoma	5.5	CD8+	5.3
28	48	Sulochana s	1062/19	IP-12644	Cystic, irregular , bossilated	Low	Serous carcinoma	6.17	CD8+	6.08

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29	40	Indirabai b	355/21	IP-12577	Cystic, glistening, pale white	Low	Serous carcinoma	7.5	CD8+	6
30	35	Annapurna	2084/21	IP-12796	Cystic, irregular , bossilated	Low	Serous carcinoma	6.8	CD8+	5.8
31	51	Chandravati p	2270/22	IP-12678	Cystic, pale white	High	Serous carcinoma	4.3	CD8+	4.46
32	45	Mahadevi languti	6733/22	OUT-100292	Cystic, glistening, gray white areas	High	Serous carcinoma	4.68	CD8+	4.63
33	42	Bharati ashok	3241/22	IP-127908	Cystic, irregular , bossilated	Low	Serous carcinoma	6.34	CD8+	5.31
34	54	Mehreen	4370/22	OUT-00085137	Multicystic, glistening, pultaceous material	High	Serous carcinoma	5.3	CD8+	4.6
35	45	Suma nagane	1636/18	IP-126352	Cystic, pale white	Low	Serous carcinoma	6.36	CD8+	5.94
36	50	Shamabai kallur	2254/21	IP- 17965	Cystic, irregular , bossilated	Low	Serous carcinoma	6.8	CD8+	6.53
37	38	Kamalabai badagar	4734/22	OUT-87087	Globular, solid, pale yellow varigated	High	Serous carcinoma	3.96	CD8+	4.29
38	42	Rajashree b	1066/21	IP- 73265	Cystic, pale white	Low	Serous carcinoma	5.8	CD8+	6.3
39	45	Shantabai rathod	2884/17	IP - 78603	Cystic, pale white, hemorrhagic	-	Endometrioid Carcinoma	5.4	CD8+	--
40	51	Sumitra bai	4508/19	IP- 06354	solid, pale white	-	Endometrioid Carcinoma	5.1	CD8+	--

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