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# Ki-67 Immunostaining in Epithelial Ovarian Tumors with Clinicopathological Correlation

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## ABSTRACT

***Background and Objective:*** *Ovarian cancer is the 8th most common gynecological cancer causing over 15,000 deaths globally. Surface Epithelial Ovarian Cancer (SEOC) comprises different ovarian tumors differing in histopathology and prognosis. Since Ki-67 is economical and reliable, hence it can be employed for assessing tumor behavior, its progression, prognosis, and evaluation of therapeutic response. This study aimed to study the Ki-67 expression in benign, borderline, and malignant SEOC along with its correlation with clinicopathological parameters.* ***Methods:*** *The prospective study included 55 patients having benign, borderline, and malignant tumors who came for routine histopathological evaluation. Tumor histopathology and Ki-67 expression were studied using immunohistochemistry and correlated with the relevant findings. Statistical analysis has been performed with the help of R i386 3.6.3. Continuous variables were given in mean ± standard deviation. Co-relation between the groups was done by Spearman correlation. p-value less ≤ 0.05 indicated significance.* ***Results:*** *Mean age of study subjects observed was 45.11 ± 13.73 (in years) with benign (36.36%), borderline (27.27%), and malignant tumors (36.36%). Serous tumors expressing high Ki-67 indicated increased frequencies of malignant tumors (64.55%). The study depicted a statistically significant correlation between Cancer Antigen 125 (CA125) levels, gross appearance, necrosis, hemorrhage, papillary projections capsular breach, malignant cytology, microscopic findings, tumor stage, and Ki-67 expression (p<0.005).* ***Conclusion:*** *The study infers that Ki-67 expression can be an aiding tool in the clinicopathological diagnosis of SEOC pointing toward the aggressiveness and proliferative nature of the cancerous cells.*

**Keywords:** Epithelial ovarian cancer, Ki 67, Ovarian tumors, Prognosis, Serous tumors

## INTRODUCTION

Ovarian Cancer (OC) has caused death in more than 15,000 women around the world. It happens to be the 8th most common gynecological cancer having a rate of survival below 45% [1]. As per the recent report of the National Cancer Registry Programme, OC is the 3rd most frequently occurring cancer in Indian women [2]. OCs are more deadly than any other female cancer due to the absence of signs and symptoms, non-specificity, and relationship with the progressive stage of the disease beyond the pelvis during diagnosis and recurrence [3].

Ovarian tumors are divided depending upon the source of origin and their histomorphological features by the World Health Organization, which are mostly of epithelial origin. Surface epithelial cancers consist of varying degrees of lesions accounting for 2/3rd ovarian neoplasms [4]. The tumors originating from the surface epithelium are mucinous, serous, clear cell, endometrioid, and Brenner (transitional cell) carcinoma. They are further sub-classified into benign, borderline, and malignant tumors [5]. Factors responsible for this ovarian carcinogenesis are estrogen-only pills, reproductive health, past OC incidences of family members, age, lynch syndrome, medical disease history, etc. [6].

Benign as well as malignant are two subgroups, which have clear-cut diagnostic findings in histopathological sections. The borderline group with atypia shows only some amount of multiplication [1,7,8]. Evaluation of cell proliferation status is critical for identification and prognosis for a better understanding of clinical behavior and severity of OC [3].

Ki-67 has been identified as a clinically important proliferation marker for indicating tumor aggression and metastasis. Monoclonal Ki-67 reacts with nuclear Ki-67 antigen found in the proliferating cells [9,10]. Expression levels of this immune-histochemical marker, possess the tumor proliferation, microscopic tumor staging, and treatment modalities responses with disease outcome prediction in many malignant diseases [4,11,12].

Tumors malignant in nature are more highly invasive as compared to benign and borderline tumors. Due to this, malignant tumors show high Ki-67 levels, while the latter shows little to no Ki-67 *via.* immunostaining [3,8,13,14]. Ovarian tumors are heterogeneous with variable degrees of disease progression. Early detection of tumors is critical for the prevention of spread in ovaries as well as timely application of chemotherapy for malignant tumors [15,16]. Ki-67 being reliable, cost-effective and a new technique helps in evaluating the proliferative index of neoplastic lesions [17,18]. Ki-67 expression marker should be preferred over other Immunohistochemistry markers (estrogen receptors, progesterone receptors, p53 gene, etc.) because of its strong correlation with the histological staging/ grading as per a study by Verma, et al. [4]. Hence, we analyzed the clinical significance between Ki-67 expression and clinicopathological factors in routine use and to further conclude if its regular use can facilitate further prognosis of the disease.

## MATERIALS AND METHODS

The prospective study has been carried out in the department of pathology from July 2016-June 2018. A total of 55 patients were included in this study ranging from 21 years to 73 years with benign, borderline, and malignant tumors who came for routine histopathological evaluation. Case files were accessed for gathering the clinical history followed by investigation details. Subjects with OC apart from the epithelial origin, with ovarian non-neoplastic lesions and ovarian tumors, which have undergone torsion or complete necrosis, were excluded from the study. The ethical approval was obtained from the Institutional research and ethics committee (SS-1/EC/04/2016). Written informed consent was obtained from the study subjects before the commencement of the study.

### Sample Size Calculation

Using G-power, with effect size d=0.42, 90% confidence level, and 80% power, the minimum sample size for each group was 15.

### Data Collection

The patients gave their informed consent, after which the required clinical details were obtained. Clinical history including patients' age and other investigational details (ultrasound and CT scan of abdomen and pelvis and Cancer Antigen 125 (CA-125) levels) were collected from the patient's case files. Specimens were received in the Pathology Department in the fresh state when a frozen section report was required, otherwise in 10% formalin. The standard protocol for surgical grossing of the ovarian specimen was followed throughout the study [19]. The specimen was fixed for 24 hours using 10% formalin. Following a detailed macroscopic description of the external surface and cut surface findings, the representative areas were sampled. After conventional processing in a Leica 1020 model histokinette and embedding in paraffin wax, sections of 5 µm thicknesses were cut using Leica JUNG RM 2025 model rotator microtome and stained using Hematoxylin and Eosin (H&E) for histopathological study. In addition, 4 µm sections were halved from a paraffin block of tumor tissue and taken on glass slides coated with poly-L-lysine for Immunohistochemistry (IHC) staining for detecting Ki-67 expression levels.

### Processing for Immunohistochemistry

Tumor tissue sections of 4 µm were taken on the glass slide for Immunohistochemistry (IHC) staining to detect Ki-67 expression. The technique for IHC included antigen retrieval in citrate buffer in a microwave oven which blocks the endogenous peroxidase with 3% hydrogen peroxide. Following incubation with primary mouse monoclonal antibody against Ki-67 protein (Biogenex), linking with rabbit anti-mouse secondary antibody (Biogenex), enzyme labeling with streptavidin-horseradish peroxidase, developing chromogen with Deaminobenzidine (DAB) and counterstaining with hematoxylin. Positive and negative controls were included in every batch. The H&E-stained slides were considered for tumor nature, histologic type, histologic grade, staging, and other features as per the standard reporting protocol. The immunostained slides were analyzed for nuclear staining with the anti-Ki-67 antibody. The ratio of Ki-67 expressing tumor cells was documented in percentile for every case.

Association of variables like age, clinical presentation, tumor size, nature, tumor histologic type, histologic grade, involvement of lymph node, tumor stage, and Ki-67 expression levels was studied.

**Statistical Analysis**

Descriptive data analysis has been performed with the help of R i386 3.6.3. Continuous variables were represented by mean ± standard deviation form. Comparison of categorical variables was carried out by chi-square test. Stimulation was done if the expected cell count is less than 5. Continuous data were compared using t-test/ANOVA with Tukey's HSD as post hoc/Kruskal Wallis test with Dunn test as post hoc. Spearman correlation has been employed to find an association between two continuous non-normal data. p-value less than or equal to 0.05 indicated significance.

## RESULTS

The study comprised of total 55 subjects with a mean age of 45.11 ± 13.73 (in years). On examination, it was noted that 90% benign and 100% borderline were unilateral tumors. While 35% of the subjects had malignant bilateral tumors. These findings depicted that the correlation between tumor laterality and tumor nature is not statistically significant. Among the total cases, 83.64% of subjects had a unilateral ovarian tumor and 16.36% had a bilateral tumor. Mucinous histology with stage I (IA, IB, IC) had the highest number of unilateral incidences in comparison with other histological types like clear cell, serous, etc. (12 cases). Serous histological types including Stage III (IIIA, IIIB, IIIC) had 3 cases of bilateral cases.

The most common clinical findings observed in these patients include pain (80%) followed by mass (55%) and menstrual irregularities (15%). Patients also had complaints of constipation (13%), breathlessness (13%), dysuria (11%), and vomiting (6%). Clinical finding of pain and mass was most commonly associated with the nature of benign (70%), borderline (87%), and malignant tumors (85%). Menstrual irregularity (35%) was another common clinical finding in benign tumors.

The mean ± SD of the CA 125 level was 336.06 ± 725.41 U/ml. There was a significant difference between the mean of CA 125 levels and the different nature of tumors. The findings also emphasized the evident differences between the mean CA 125 levels of malignant tumors as compared to that of benign and borderline tumors ($p=0.0030$) respectively. The correlation between gross appearance and the tumor nature showed statistical significance ($p<0.0005$). The commonest gross finding in benign tumors was their cystic nature (50%). Borderline tumors were predominantly solid and cystic with thick septations (53.3%) and solid and cystic (65.5%) findings in the malignant tumors were commonly found. The smallest ovarian tumor was 2.7 cm across and the largest tumor measured 36 cm across. The mean size of the ovarian tumors studied was 14.67 ± 7.79 cm and the median size was 13.6 cm. The mean tumor size in the case of benign, borderline, and malignant tumors was 11.16 cm (SD ± 6.43), 20.06 cm (SD ± 7.63), and 14.14 cm (SD ± 7.20) respectively.

The Ki 67 was compared over the tumor nature and showed that the distribution of Ki 67 was significantly different between at least 2 and a maximum of 3 tumor nature ($p<0.0001$ for each). There were 11 benign cases (55%) having Ki-67 expression less than 1% categorized as negative or group 0. Positive groups (≥ 1% tumors immunostained with Ki-67) were further divided into groups 1 to group 4. Benign mucinous tumors overall were placed in negative or group 1. Borderline tumors predominantly (90%) showed group 2 Ki-67 expressions and malignant tumors ranged from group 2 to group 4. It was shown that Ki-67 was significantly associated with the histologic grade of the tumor (Table 1).

**Table 1 Ki-67 group distribution in relation to serous, mucinous tumors, and histologic grade**

| Serous Tumors | Negative (<1%) | 1 (1%-5% Positive) | 2 (6%-30% Positive) | 3 (31%-50% Positive) | 4 (>51% Positive) | Total |
|---|---|---|---|---|---|---|
| Benign | 6 (60%) | 4 (40%) | 0 (0%) | 0 (0%) | 0 (0%) | 10 |
| Borderline | 0 (0%) | 0 (0%) | 5 (100%) | 0 (0%) | 0 (0%) | 5 |
| Malignant | 0 (0%) | 0 (0%) | 1 (7.7%) | 2 (15.4%) | 10 (76.9%) | 13 |

| **Mucinous Tumors** | **Negative (<1%)** | **1 (1%-5% Positive)** | **2 (6%-30% Positive)** | **3 (31%-50% Positive)** | **4 (>51% Positive)** | **Total** |
|---|---|---|---|---|---|---|
| Benign | 1 (33.3%) | 2 (66.7%) | 0 (0%) | 0 (0%) | 0 (0%) | 3 |
| Borderline | 0 (0%) | 1 (10%) | 9 (90%) | 0 (0%) | 0 (0%) | 10 |
| Malignant | 0 (0%) | 0 (0%) | 2 (40%) | 1 (20%) | 2 (40%) | 5 |
| **Histological Grade** | **Ki-67 Groups** | **Histologic grade of tumor** | | | | **p-value** |
| | | **G1** | **G2** | **G3** | **GB** | |
| | 1 (1%-5% Positive) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (100%) | 0.0150*[MC] |
| | 2 (6%-30% Positive) | 0 (0%) | 3 (17.6%) | 0 (0%) | 14 (82.4%) | |
| | 3 (31%-50% Positive) | 1 (33.3%) | 1 (33.3%) | 1 (33.3%) | 0 (0%) | |
| | 4 (>51% Positive) | 0 (0%) | 13 (92.9%) | 1 (7.1%) | 0 (0%) | |
| [MC]: indicates chi-square test with simulation; * indicates statistical significance | | | | | | |

The study showed association of age and CA125 levels with Ki 67 is statistically significant. A significant co-relation of different Ki-67 expression groups with size categories was also observed (p-value=0.0395).

Gross appearance and Ki-67 subdivisions showed a significant association expression of the tumors (p=0.0015). The negative group had predominantly a cystic appearance (72.7%). The low and high proliferation groups showed solid with the cystic appearance of (48.1%) and (94.1%) (Table 2).

**Table 2 Gross appearance of the tumors and Ki-67 subdivisions**

| **Ki-67 Subdivision** | **Gross appearance** | | | **p-value** |
|---|---|---|---|---|
| | **Solid** | **Cystic** | **Solid and cystic** | |
| Negative | 0 (0%) | 8 (72.7%) | 3 (27.3%) | 0.0015*[MC] |
| Low proliferation (group 1 and 2) | 1 (3.7%) | 13 (48.1%) | 13 (48.1%) | |
| High proliferation (group 3 and 4) | 0 (0%) | 1 (5.9%) | 16 (94.1%) | |
| [MC]: indicates chi-square test with simulation, * indicates statistical significance | | | | |

These high and low proliferation groups showed significant differences with respect to necrosis and hemorrhage (p<0.005) (Table 3).

**Table 3 Necrosis, hemorrhage, and KI-67 proliferation group**

| **Ki-67 Subdivision** | **Necrosis** | | **p-value** |
|---|---|---|---|
| | **Present** | **Absent** | |
| Negative | 0 (0.0%) | 11 (100%) | 0.0005* [MC] |
| Low Proliferation (Group 1 and 2) | 4 (14.8%) | 23 (85.2%) | |
| High Proliferation (Group 3 and 4) | 14 (82.4%) | 3 (17.6%) | |
| | **Hemorrhage** | | |
| | **Present** | **Absent** | |
| Negative | 1 (9.1%) | 10 (90.9%) | 0.0015*[MC] |
| Low Proliferation (Group 1 and 2) | 5 (18.5%) | 22 (81.5%) | |
| High Proliferation (Group 3 and 4) | 11 (64.7%) | 6 (35.3%) | |
| [MC]: indicates chi-square test with simulation, * indicates statistical significance | | | |

Papillary projections, capsular breach, and malignant cytology with the Ki-67 groups were significantly associated ($p<0.05$) (Table 4).

**Table 4 Ki-67 groups with respect to papillary projections, capsule, and malignant cytology**

| Ki-67 Groups | Papillary Projections | | p-value |
|---|---|---|---|
| | Present | Absent | |
| Negative (<1%) | 1 | 10 | 0.0045* MC |
| 1 (1%-5% Positive) | 3 | 7 | |
| 2 (6%-30% Positive) | 6 | 11 | |
| 3 (31%-50% Positive) | 1 | 2 | |
| 4 (>51% Positive) | 11 | 3 | |
| | Capsule | | |
| | Intact | Breach | |
| Negative (<1%) | 11 | 0 | 0.0085* MC |
| 1 (1%-5% Positive) | 10 | 0 | |
| 2 (6%-30% Positive) | 15 | 2 | |
| 3 (31%-50% Positive) | 3 | 0 | |
| 4 (>51% Positive) | 8 | 6 | |
| | Malignant Cytology | | |
| | Present | Absent | |
| Negative (<1%) | 0 (0%) | 11 (100%) | 0.0010* MC |
| 1 (1%-5% Positive) | 0 (0%) | 10 (100%) | |
| 2 (6%-30% Positive) | 2 (11.8%) | 15 (88.2%) | |
| 3 (31%-50% Positive) | 1 (33.3%) | 2 (66.7%) | |
| 4 (>51% Positive) | 8 (57.1%) | 6 (42.9%) | |
| MC: indicates chi-square test with simulation, * indicates statistical significance | | | |

Lymph node metastases were observed in four of the total 20 malignant cases studied, of which two were serous carcinomas of high grade, one mucinous, and one clear cell carcinoma. Metastatic lymph nodes in 75% of cases had a high Ki-67 expression. However, no significant correlation between lymph node metastases and Ki-67 expressions ($p=0.1839$) was noted.

Among the total 35 borderline and malignant cases taken, 13 borderline and 8 malignant were of FIGO stage I disease, two borderline and four malignant were in stage II, and 7 and 1 malignant were in stage III and stage IV respectively (Table 5).

**Table 5 Ki-67 groups with respect to tumor stage**

| Ki-67 Groups | Stage | | | | Total |
|---|---|---|---|---|---|
| | Stage I (IA, IB, IC) | Stage II (IIA, IIB) | Stage III (IIIA, IIIB, IIIC) | Stage IV | |
| 1 (1%-5% Positive) | 1 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 |
| 2 (6%-30% Positive) | 13 (76.5%) | 3 (17.6%) | 1 (5.9%) | 0 (0%) | 17 |
| 3 (31%-50% Positive) | 1 (33.3%) | 0 (0%) | 2 (66.7%) | 0 (0%) | 3 |
| 4 (>51% Positive) | 6 (42.9%) | 3 (21.4%) | 4 (28.6%) | 1 (7.1%) | 14 |
| **Ki-67 SUBDIVIONS** | | | **Stage I+II** | **Stage III+IV** | **Total** |
| Low proliferation group (group 1 and 2) | | | 17/18 | 1/18 | 18 |
| High proliferation group (group 3 and 4) | | | 10/17 | 7/17 | 17 |

When low and high proliferation groups of Ki-67 were compared to the stages of a tumor, it was seen that there was an association between the groups with a p-value of 0.0170, which was statistically significant (Table 5).

## DISCUSSION

OCs are inexplicable diseases due to complex pathogenesis and constant variations in the ovaries, susceptible to neoplastic and non-neoplastic lesions. Identification of such proliferative activity is a must. Ki-67 is one such proliferative marker that helps in identifying varied ovarian malignancy with a fraction of the growth and aggressiveness of tumor cells. The present study had the majority of patients in the 3rd and 4th decade of life with a mean age of 45.11 years. There was no association of age to tumor nature, similar to the findings by Kaur J, et al. amongst all the patients, 83.46% had unilateral tumors, matching to findings of Mahadevappa, et al. with 65.5% of unilateral tumors [3,20]. Among these patients 18 (90%) were benign, 15 (100%) borderline, and 13 (65%) malignant tumors [5]. On the contrary Yasmin, et al. reported the majority of benign ovarian tumors (89.71%) and the least of malignant tumors (10.29%) [21]. Mucinous histology with FIGO stage I (IA, IB, IC) had the maximum number of unilateral cases followed by serous histological type with three bilateral cases in the present study. Similar to results by Fischerova D, et al., this showed a majority of the OC were of serous histotype, subsequent with mucinous histotype which is very rare [22].

It is of utmost importance that these SEOCs along with their grade are detected early. Proliferative markers are essential for prognosis in ovarian tumor patients. Our study used the Ki-67 expression as a marker for detecting the severity of OET (benign, borderline, and malignant). The average Ki-67 Labeling Index (LI) was 0.99% in benign, 10.83 is borderline, and 64.55% in malignant tumors showing a statistical significance ($p<0.05$). This difference was attributed to the nuclear factor involved in the progression of tumor growth, which indeed reflected the Ki-67 expression. As the tumor grows, the Ki-67 expression also changes. The findings were similar to a study by Sardar K, et al. where values of malignant tumors were higher [23]. Our study depicted a statistically significant association with the histological grade. Among the 20, 17 carcinomas we studied were moderately differentiated, G2 (82%), and 2 were poorly differentiated. The results were compared with the findings by Korkolopoulou P, et al. were, 39 out of 80 cases were moderately differentiated (48.75%), 27 poorly differentiated (33.75%), and the rest were well differentiated [24]. The study also showed statistical significance between CA125 levels and the Ki-67 expression ($p<0.0001$). This has indicated that higher the CA125 levels, there are higher chances of the malignancy of tumors. Similar results were observed by Laishram, et al. which signifies a positive co-relation of CA125 with Ki-67 expression levels ($p=0.008$) [25].

Our study observed a positive association between the gross appearance and Ki-67 proliferation groups. Tumors of high proliferative groups had 94% solid with cystic appearance, 65.7% of hemorrhage, and necrosis (82.4%). Thus, we can imply that predominantly solid with cystic tumors with areas of necrosis and hemorrhage shall have a higher proliferation group or a higher Ki-67 LI. The papillary projections can be linked to high Ki-67 expression. This is because cells of higher proliferation affect the clinical pattern and aggressiveness of the OC, with high expression of Ki-67 [3]. Thus in our study out of the 10 serous carcinomas 8 were of high-grade Ki-67 expression. A study by Ramlingam P, et al. stated that this type of High-Grade Serous Carcinoma (HGSC) of the papillary pattern is linked with a high Ki-67 expression (value=96%) [26]. A high number of malignant tumors presenting with capsular breach were observed. The majority were HGSC, which indicated Ki-67 expression at a greater rate. High values of Ki-67 show increased tumor growth and impendence of capsular breach showing a poor outcome. Related findings were noticed for malignant cytology. There was no association of the metastasis of lymph node with Ki-67 expression alike similar to the findings by Martins, et al. Metastasis of lymph node involvement of malignant HGSC was seen only in two cases, despite being a high potential for lymph node metastasis [27].

## CONCLUSION

The study findings conclude that Ki-67 expression would help in the clinic-pathological diagnosis of SEOC. Since Ki-67 is an economical and reliable tool, it could be employed in the determination of the tumor behavior, its progression, prognosis, and assessment of therapeutic response.

## DECLARATIONS

### Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## REFERENCES

[1] Webb, Penelope M., and Susan J. Jordan. "Epidemiology of epithelial ovarian cancer." *Best Practice & Research Clinical Obstetrics & Gynaecology,* Vol. 41, 2017, pp. 3-14.

[2] Takiar, Ramnath. "Status of ovarian cancer in India (2012-14)." *EC Gynaecology,* Vol. 8, No. 5, 2019, pp. 358-64.

[3] Mahadevappa, Asha, Shruthi Mysore Krishna, and Manjunath Gubbanna Vimala. "Diagnostic and prognostic significance of Ki-67 immunohistochemical expression in surface epithelial ovarian carcinoma." *Journal of Clinical and Diagnostic Research: JCDR,* Vol. 11, No. 2, 2017, pp. 8-12.

[4] Verma, R., et al. "Histological grade, CA-125 Levels and IHC expression of ER/PR, HER-2/NEU, p53 and Ki-67 markers in epithelial ovarian neoplasms: A correlative study." *International Journal of Advanced Research,* Vol. *5*, No. 6, 2017, pp. 235-54.

[5] Modepalli, Nalini, and Suguna Belur Venugopal. "Clinicopathological study of surface epithelial tumors of the ovary: An institutional study." *Journal of Clinical and Diagnostic Research: JCDR,* Vol. 10, No. 10, 2016, pp. EC01-04.

[6] Zayyan, Marliyya S. "Risk factors for ovarian cancer." *Tumor Progression and Metastasis*, Vol. 8, 2020, pp. 75-102.

[7] Zhang, Shuang, et al. "Diagnostic extended usefulness of RMI: Comparison of four risk of malignancy index in preoperative differentiation of borderline ovarian tumors and benign ovarian tumors." *Journal of Ovarian Research,* Vol. 12, No. 1, 2019.

[8] Seidman, Jeffrey D., et al. "Surface epithelial tumors of the ovary." *Blaustein's Pathology of the Female Genital Tract*, New York, NY: Springer, 6th ed., 2011.

[9] Gursan, Nesrin, et al. "P53, bcl-2, ki-67 li (labeling index) status in benign, proliferative, and malignant ovarian surface epithelial neoplasms." *The Eurasian Journal of Medicine,* Vol. 41, No. 1, 2009, pp. 10-14.

[10] Remnant, Lucy, et al. "The intrinsically disorderly story of Ki-67." *Open Biology,* Vol. 11, No. 8, 2021.

[11] Mita, Shunji, et al. "Prognostic significance of Ki-67 antigen immunostaining (MIB-1 monoclonal antibody) in ovarian cancer." *Journal of Nippon Medical School,* Vol. 71, No. 6, 2004, pp. 384-91.

[12] Singh, Mahendra, Lubna Khan, and Ankita Kamthan. "Immunohistochemical expression of Ki-67 and p53 in surface epithelial ovarian tumor." *Journal of Evidence Based Medicine and Healthcare,* Vol. 8, No. 18, 2021, pp. 1241-45.

[13] Dewi, I. Gusti Ayu Sri Mahendra, Anak Agung Ayu Ngurah Susraini, and Ni Putu Ekawati. "HER2/neu and Ki-67 as prognostic factors in serous type ovarian carcinoma." *Bali Medical Journal,* Vol. 9, No. 2, 2020, pp. 567-71.

[14] Basak, Bijan, Santosh Kumar Mondal, and Sharmistha Debnath. "WT1, Bcl-2, Ki-67 and Her2/Neu as diagnostic and prognostic immuno markers in ovarian serous and endometrioid carcinoma." *International Journal of Clinical and Diagnostic Pathology,* Vol. 3, No. 3, 2020, pp. 153-57.

[15] Choudhury, Monisha, Seema Goyal, and Mukta Pujani. "A cytohistological study of Ki-67 expression in ovarian tumors." *Indian Journal of Pathology and Microbiology,* Vol. 54, No. 1, 2011, pp. 21-24.

[16] Trinidad, Camille V., et al. "Reducing ovarian cancer mortality through early detection: Approaches using circulating biomarkers." *Cancer Prevention Research,* Vol. 13, No. 3, 2020, pp. 241-52.

[17] Li, Lian Tao, et al. "Ki67 is a promising molecular target in the diagnosis of cancer." *Molecular Medicine Reports,* Vol. 11, No. 3, 2015, pp. 1566-72.

[18] ALgarsh, Hassan, Hana Abusaida, and Fairouz Torjman. "The correlate between argyrophilic nucleolar organizer region stain and Ki-67 immnochistochemistry in diagnostic breast cancer." *Iberoamerican Journal of Medicine,* Vol. 2, No. 4, 2020, pp. 285-92.

[19] Malpica, Anais, et al. "Endometrial carcinoma, grossing and processing issues: Recommendations of the International Society

of Gynecologic Pathologists." *International Journal of Gynecological Pathology,* Vol. 38, No. 1 Suppl 1, 2019, pp. S9-S24.

[20] Kaur, Jasmine, et al. "Ovarian neoplasms: Histopathological patterns and estrogen and progesterone receptor expression in epithelial ovarian tumors." *Annals of International Medical and Dental Research,* Vol. 3, No. 3, 2017, pp. 33-37.

[21] Yasmin, Sumaira, Aiman Yasmin, and Mohammad Asif. "Clinicohistological pattern of ovarian tumors in Peshawar region." *Journal of Ayub Medical College Abbottabad,* Vol. 20, No. 4, 2008, pp. 11-13.

[22] Fischerova, Daniela, et al. "Diagnosis, treatment, and follow-up of borderline ovarian tumors." *The Oncologist,* Vol. 17, No. 12, 2012, pp. 1515-33.

[23] Sardar, Kumudini, Jitendra Singh, and Sarita Tirkey. "Ki67 immuno-expression in ovarian tumors." *IOSR Journal of Dental and Medical Sciences,* Vol. 17, No. 2, 2018, pp. 34-37.

[24] Korkolopoulou, Penelope, et al. "The combined evaluation of p27Kip1 and Ki-67 expression provides independent information on overall survival of ovarian carcinoma patients." *Gynecologic Oncology,* Vol. 85, No. 3, 2002, pp. 404-14.

[25] Laishram, Sheronica, et al. "To assess the utility of proliferative marker Ki-67 in surface epithelial ovarian tumor." *Journal of Datta Meghe Institute of Medical Sciences University,* Vol. 14, No. 1, 2019, pp. 6-10.

[26] Ramalingam, Preetha. "Morphologic, immunophenotypic, and molecular features of epithelial ovarian cancer." *Oncology (Williston Park, NY),* Vol. 30, No. 2, 2016, pp. 166-76.

[27] Martins, Sandra F., et al. "Ki-67 expression in CRC lymph node metastasis does not predict survival." *BioMed Research International,* Vol. 2015, 2015.