International Journal of Biotechnology and Clinical Medicine ISSN 2583-4665
Vol.2, No.4, Oct - Dec 2023, pp. 08~13

KI 67 ANTIGEN EXPRESSED BY BENIGN, BORDERLINE, AND OVARIATIC CARCINOMAS

AM. Chandrika[1], G. Jayalakshmi[1*]
[1]Department of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry - 605502, India.

Article Info

Article history:

Received November 11th, 2023
Revised November 29th, 2023
Accepted December 15th, 2023

Keyword:

Tumor science
Cancer biology
Cystic degeneration
Oxidative stress
Proliferation

ABSTRACT

To compare the Ki 67 expression in benign borderline and malignant ovarian tumors and study the statistical significance of Ki67 expression and its correlation with tumor stage and type. First standard classification of ovarian tumors was proposed by P. fannensteil in the year 1898. In the year 1958, Navak proposed classification based on clinical findings, dividing them to solid and cystic varieties. Thus the borderline tumors, solid tumors with cystic degeneration and predominantly cystic tumors with solid areas fall into grey zone. In the year 1973 WHO classification of ovarian tumors based on the histogenesis was made. This classification system was updated in 2014 and was approved by the international society of gynaecological pathologists.

Corresponding Author:

Dr. G. Jayalakshmi,
Department of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry - 605502, India.
Email: jayalakshmi.2k15@gmail.com

How to Cite:

Chandrika AM and Jayalakshmi G. Ki 67 antigen expressed by benign, boderline, and ovariatic carcinomas. IJBTCM. 2023; Volume 2 (Issue 4): Page 08-13.

1. INTRODUCTION

In India, ovarian malignancies are the second most common after cervical cancer [1]. In Chennai ovarian tumors stands at 3rd position among malignancies in women with an incidence of 6 cases per 100000 women [2]. Ovarian cancer is the 7th most common cancer in women Worldwide [3]. Despite improvements in our understanding of the illness over the past several decades, ovarian tumours nevertheless provide a challenge [4]. They represent a whole range of neoplasms, ranging from specialised hormone-secreting cells to germinal and embryonal cells [5]. The prognosis of patients with ovarian cancers is greatly influenced by the tumour stage, postoperative residual tumour burden, histology type, and histological grade [6].

Excessive and uncontrolled cellular proliferation is one of the characteristics of malignancies [7]. Mitotic count is an age old method to determine the proliferative activity in histopathological sections of breast, neural tumors etc., but the results can be variable due to inter observer variation [8]. Alternatively, immunohistochemical detection of proliferating cells may help to determine the proliferation of tumor cells in a more accurate way. Ki 67 is one such Proliferative marker [9]. Ki 67 antigen is expressed during all active phases of the cell cycles except in resting phase 6. Monoclonal Ki 67 antibody (MIB-1) binds to the Ki 67 nuclear antigen. Utility of Ki 67 as a proliferative marker is well established in tumors of breast and new tissues where the high expression of Ki 67 indicates poor prognosis. The role of Ki 67 as a reliable prognostic factor in ovarian tumors is being studied actively throughout the world. But in India very few workers have conducted study in this aspect, thus highlighting the need for further investigation and research.

Hemophilus of Chalcedon, a renowned anatomist of the Alexandrian School who lived in the fourth century B.C., should be the starting point of any history of the ovary. Hemophilus is recognised as the first anatomist to describe mammalian ovaries [10]. "Female testis," he referred to it as. By the fifth week of pregnancy, the ovum begins to develop. Thickenings of the lining of the posterior embryonic body cavity, which later extend into the mesenchyme beneath, result in the development of the primordial gonads. Primitive gonads were identified as

an ovary at two months of gestation because the testicular sex cords had not yet fully developed. Incomplete ovarian sex cords that are embedded in primitive mesenchyme are still formed by mesonephric cells and germ cells that are still intimately linked. The coelomic epithelium is still present in the ovary's perimeter and is encircling it [11].

The ovary is lined by a single layer of cells which originates from coelomic epithelium, also known as germinal epithelium or surface epithelium. The cells can be flattened, cuboidal, columnar or focally pseudostratified [12]. The ovanan stroma is divided into cortex and medulla. The cortex can be generally divided into an inner, more active cortex and an outside, fibrous, acellular collagenous zone known as the "Tunica albuginea." The primordial follicle, npenmg follicle, and mature follicles are all within. The centre region of the ovary is called the medulla, and it also comprises active follicles and cellular stroma. The stroma is made up of uniformed spindle cells arranged in bundles, frequently in storiform pattern [13].

The hilum region is where the blood vessels and a tiny quantity of connective tissue enter. The ovarian hilar area and neighbouring mesoovarium include the hilus cell nests, which are uncapsulated aggregates of varying sizes [14]. These hilus cells feature a vesicular nucleus, a lot of eosinophilic cytoplasm, one or two nucleoli, and a range of shapes from round to oval. Hilus cells display specific Reinke crystals. Homogenous are eosinophilic, non-refractile rod-shaped forms. A lifelong supply of eggs are available in the primordial follicles in the ovaries at birth, but most of these follicles suffer atresia during infancy, and only 400 of these follicles are present by adolescence. During puberty, the hypothalamus starts a pulsatile cycle.

2. MATERIALS & METHODS

This is a prospective study conducted m the Department of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences and Hospital, from June 2019 to July 2020. During the study period of 18 months, 86 ovarian neoplasms were received, which were included in the study after excluding the non-neoplastic lesions. This study was approved by the ethical committee of the institution.

2.1. MATERIAL FOR THE STUDY

The material for this study was received as surgically resected specimens from the patients admitted in Sri Lakshmi Narayana Institute of Medical Sciences and Hospital, who underwent oophorectomy or salpingo-oophorectomy or ovariotomy or total hysterectoy.

2.2. INCLUSION CRITERIA

Benign, borderline and malignant ovarian neoplasms.

2.3. EXCLUSION CRITERIA

Non-neoplastic lesions of the ovary like follicular cyst, hemorrhagic cyst, torsion ovary, ectopic pregnancy, etc.

2.4. METHOD OF THE STUDY

Clinical details like age, obstetric history, clinical signs and symptoms were entered in the proforma. Thorough gross examination was done and features such as size, external appearance, consistency, findings on cut section and contents were noted, if the tumor was cystic. Depending on the size and consistency of the tumors were cut at various levels to allow proper fixation in 10% NBF for 24 to 48 hours. After adequate fixation, multiple bits were taken. Multiple bits were taken from representative areas. The bits acquired were processed in automated tissue processor. The processed tissues were embedded manually and paraffin wax blocks were made. The blocks were cut at 4μ thickness and mounted onto albumin coated glass slides which are later de-waxed and routinely stained with Hematoxylin and eosin stain. PAS stain was done m mucinous cystadenocarcinomas and krukenberg tumors. A detailed microscopic examination of the tumor was done to arrive at the histopathological diagnosis according tothe WHO classification of ovarian tumors, the grade of the tumor and staging of the tumor was reported where ever possible.

2.5. IMMUNOHISTOCHEMICAL EVALUATION

The immunohistochemical detection of biomarker in the ovarian tumors were conducted using monoclonal primary antibody (anti Ki67) against Ki67 nuclear antigen. Ki67 immunostaining was done using peroxidase- anti-peroxidase technique in benign (63), borderline (4) and malignant (19) ovarian neoplasms.

2.6. STAINING PROCEDURES HEMATOXYLIN AND EOSIN

1. To deparaffinise slides were immersed in first xylene bath for 3 minutes.
2. Then transferred to second xylene bath for 2-3 minutes.
3. Immersed in 70% alcoh] bath for 3 minutes.
4. Rinsed in running water for 1minute and briefly 1n distilled water.
5. Stained with Harri's haematoxylin for 5-10 minutes and rinsed in tap water.
6. Differentiated in 1% acid alcohol by dipping 3-4 times and washed in tap water briefly.
7. Blueing was done with lithium carbonate, sections appeared blue.
8. Rinsed in tap water for 10-20 minutes.
9. Stained with acidified 1% aqueous eosm for 30 seconds
10. Washed in running tap water for 1 minute.
11. Dehydrate by passing through 3 baths of alcohol.
12. Passed through xylene for 15-20 seconds.
13. Mounted in DPX.

2.7. PREPARATION OF SCHIFF REAGENT

Dissolve 1.9gm of sodium meta bisulfite and 1gm of basic fusion are combined with 100ml of 0.15N hydrochloric acid. Shake the mixture intermittently till it turns clear and changes colour from yellow to light brown. Shake for one to two minutes while adding 500mg of activated charcoal. Apply a No. I Whatman filter to the solution to filter it. The filtrate ought to be transparent and colourless. Then, it is kept chilled to 4 degrees Celsius.

1. Deparaffinised in xylene and hydrated in graded ethanol to deionized water.
2. Treat (oxidized) with periodic acid for 5 minutes.
3. Wash well with several changes of distilled water.
4. Cover the sections with schiff ' s reagent for 15 minutes.
5. Wash in running tap water for 5-10 minutes.
6. Stain nuclei with Harris Hematoxylin. Differentiate In acid alcohol and blueing in tap water for 5 minutes.
7. Washed in water.
8. Dehydrated in graded ethanol solutions.
9. Clear in Xylene and mount with DPX.

2.8. IMMUNOHISTOCHEMISTRY

It is the demonstration of the antigen in the tissue sections by the use of specific immunological interations culminating m the attachment of a visible marker to the antigen. Pe roxid e, anti-peroxide (PAP) antibody method was used.

3. RESULTS

During the study period of 18 months from April 2016 to September 2017, 86 ovarian tumors were received in the department of Pathology Sree Bharath Medical College and Hospital, which were included in the study. Benign tumors were the most common tumors constituting of 63 (73.3%) cases, followed by malignant tumors with 19 cases(22%) and borderline tumors with 4 cases (4.7%).

3.1. DISTRIBUTION OF THE TYPE OF TUMOR IN RELATION TO THE TOTAL CASES

Among 86 ovarian tumors studied 55 were s u r f a c e epithelial tumors, 25 were germ cell tumors, 4 were sexcord stromal tumors and 2 were krukenberg tumors.

Table 1: Incidence of various Histological types of ovarian tumors

Histological type	No. of cases	Percentage
Surface epithelial	55	64
tumors	42	47.9

Benign Borderline Malignant	4 9	4.6 10.5
Sexcord stromal tumors Benign Malignant	4 2 2	4.6 2.3 2.3
Germ cell tumors Benign Malignant	25 19 6	29.1 22
Metastatic tumors	2	2
Total	86	100

In our study the ovarian neoplasms occurred in age group 14 to 75 years. The incidence was significantly high in the age group of 41 t0 50 years. The youngest patient was 14 years old who was diagnosed as benign mature cystic teratoma. The oldest patient was 75 years old and was diagnosed to have benign mucinous cystadenoma. Benign neoplasms were common in the age group of 21 to 30 years, borderline neoplasms were more common in the age groupof 41 to 50 years and malignant neoplasms were common during 31 to 40 years.

Table 2: Distribution of Ovarian neoplasms in differentage groups

Age in years	**Benign**	**Borderline**	**Malignant**	**No of cases**
0-20	3	-	1	4(4.7%)
21-30	18	-	3	21(24.4%)
31-40	14	1	5	20(2 3.3%)
41-50	17	2	4	23(26.7%)
51-60	6	1	2	9(10.4%)
61-70	2	-	3	5(5.8%)
70-80	3	-	1	4(4.7%)

Mean age of incidence	39.12 years	46.25 years	43.47 years	86(100%)

Table 3: Symptoms of ovarian tumors

Clinical feature	Benign	Borderline	Malignant	No of cases	Percentage
Abdominal mass	25	1	4	30	34.9%
Abdominal pam	16	1	2	19	22%
Menstrual disturbance	4	-	2	6	7%
Urinary disturbance	2	-	3	5	5.8%
Abdominal pain and mass	5	2	7	14	16.3%
Others	11	-	1	12	14%

Out of 86 cases majority of them were unilateral ovanan tumors. Microscopically the **benign** tumors showed cyst wall lined by a single layer of ciliated columnar epithelial cells with papillary projections. The cells showed no atypia. A prominent stromal component was seen in cystadeno fibromas. The mean Ki 67 index was 5%. The **borderline** tumors showed the atypical cells with hyperchromatic nuclei arranged 1n complex papillary pattern, there was no evidence of stromal invasion. The mean Ki 67 index was 26.5%. Serous **cystadenocarcinoma** showed predominantly papillary structures with fibrovascular cores, complex glands, solid sheets and islands of tumor cells with diffuse invasion of the stroma. Areas hemorrhage and necrosis were seen in 2 cases. The mean Ki 67 index was 52%. The Ki 67 index was significantly high in the malignant serous tumors and the difference was statistically significant ($p<0.001$). Microscopically, all **benign** tumors showed cyst walllined by endocervical type lining of tall columnar cells with apical mucin and basal nuclei. The mean Ki 67 index was 4.7%.

4. CONCLUSION

In 86 ovarian tumors studied over the periosd of 18 months, 73.3% were benign tumors, 22% were malignant and 4.7% were borderline ovarian tumors. The Ki 67 labeling index showed significant rise from benign to malignant ovarian tumors. As the tumor grade rises from low grade to high grade the Ki67 index rises significantly. It is also observed that the expression of Ki67 increased significantly from early stage to late stage of ovarian neoplasms. Thus the Ki67 proliferative index can be applied for ovarian neoplasms to differentiate

benign, borderline and malignant tumors. Also the high Ki67 index reflects higher clinical stage and higher grade of tumors.

FUNDING

Nil

ETHICAL APPROVAL

Nil

COMPETING INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Bharathkumar N, Sunil A, Meera P, Aksah S, Kannan M, Saravanan KM, et al. CRISPR/Cas-Based Modifications for Therapeutic Applications: A Review. Mol Biotechnol 2021;64:355–72.

[2] Viswanathan A, Musa A, Murugesan A, Vale JR, Afonso CAM, Konda Mani S, et al. Battling Glioblastoma: A Novel Tyrosine Kinase Inhibitor with Multi-Dimensional Anti-Tumor Effect (Running Title: Cancer Cells Death Signalling Activation). Cells 2019;8.

[3] Shreeyanta KC, Shah RK, Singh A, Prasai A, Bhandari B, Aryal S, et al. Prevalence of polycystic ovarian syndrome among medical students of a tertiary care hospital. J Nepal Med Assoc 2020;58:297–300.

[4] Zhang H, Li J, Saravanan KM, Wu H, Wang Z, Wu D, et al. An Integrated Deep Learning and Molecular Dynamics Simulation-Based Screening Pipeline Identifies Inhibitors of a New Cancer Drug Target TIPE2. Front Pharmacol 2021;12:772296.

[5] Sundaram KKM, Bupesh G, Saravanan KM. Instrumentals behind embryo and cancer: a platform for prospective future in cancer research. AIMS Mol Sci 2022;9:25–45.

[6] Gnanavel M, Murugesan A, Mani SK, Kandhavelu M, Yli-Harja O. Identifying the mirna signature association with aging-related senescence in glioblastoma. Int J Mol Sci 2021;22:1–14.

[7] Senthil R, Meenakshi Sundaram KK, Bupesh G, Usha S, Saravanan KM. Identification of oxazolo[4,5-g]quinazolin-2(1H)-one Derivatives as EGFR Inhibitors for Cancer Prevention. Asian Pacific J Cancer Prev 2022;23:1687–97.

[8] Ramer R, Eichele K, Hinz B. Upregulation of tissue inhibitor of matrix metalloproteinases-1 confers the anti-invasive action of cisplatin on human cancer cells. Oncogene 2007;26:5822–7.

[9] Saravanan KM, Kannan M, Meera P, Bharathkumar N, Anand T. E3 ligases: a potential multi-drug target for different types of cancers and neurological disorders. Future Med Chem 2022;14:187–201.

[10] Srimathi Devi J, Haripriya D, Arul S, Saravanan KM, Bupesh G. Evaluation of anti-cancer effect of zerumbone and cisplatin on N-nitrosodiethylamine induced hepatic cancer in freshwater fish (Danio rerio). Nat Prod Res 2021:1–5.

[11] Le HTT, Murugesan A, Ramesh T, Yli-Harja O, Konda Mani S, Kandhavelu M. Molecular interaction of HIC, an agonist of P2Y1 receptor, and its role in prostate cancer apoptosis. Int J Biol Macromol 2021;189:142–50.

[12] Nguyen P, Doan P, Rimpilainen T, Konda Mani S, Murugesan A, Yli-Harja O, et al. Synthesis and Preclinical Validation of Novel Indole Derivatives as a GPR17 Agonist for Glioblastoma Treatment. J Med Chem 2021;64:10908–18.

[13] Doan P, Nguyen P, Murugesan A, Subramanian K, Mani SK, Kalimuthu V, et al. Targeting orphan g protein-coupled receptor 17 with t0 ligand impairs glioblastoma growth. Cancers (Basel) 2021;13.

[14] Zhang H, Shao X, Peng Y, Teng Y, Saravanan KM, Zhang H, et al. A novel machine learning based approach for iPS progenitor cell identification. PLoS Comput Biol 2019;15.