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RESEARCH PAPER

Expression of Ki-67 and KRAS in Bangladeshi Ovarian Cancer Patients Attending a Tertiary Care Cancer Hospital

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Abstract

Background: Among several biomarkers Ki-67 and KRAS are two significant biomarkers providing information of biological behavior of ovarian cancer and targeted therapies. In this study the expressions status of Ki-67 and KRAS were assessed and correlated with each other and with the cancer-related characteristics.

Objective: The aim of this study was to assess and correlate the expressions of Ki-67 and KRAS in ovarian cancer patients and analyze the expressions with cancer-related characteristics of the patients.

Methods: In this cross-sectional analytical study 30 formalin-fixed paraffin-embedded (FFPE) ovarian cancer tissue blocks and 20 FFPE non-cancerous ovarian tissue blocks were collected and immunohistochemical staining for Ki-67 and KRAS expressions in these tissue sections were done. Cancer-related information of the patients were collected. Immunohistochemical staining for Ki-67 and KRAS were analyzed to assess the expression in ovarian cancer tissues and to observe the association of their expressions with each other and with the cancer-related characteristics.

Results: Expression of Ki-67 was significantly high in cancerous than non-cancerous ovarian tissues and Ki-67 index was ≥20% in 60% of ovarian cancer tissue. KRAS was expressed in 20% of ovarian cancer tissues and none of the non-cancerous ovarian tissues. Statistically significant association was found between the positive expression of KRAS with pelvic organs and lymph nodes involvement. Expression of Ki-67 and KRAS also significantly associated with each other.

Conclusion: Ki-67 was highly expressed in ovarian cancer tissue than non-cancerous ovarian tissue and Ki-67 expression was significantly associated with KRAS expression. KRAS expression is significantly associated with pelvic organs and lymph nodes metastases. So, these two markers can be new biomarkers for assessing biological behaviour and progression.

Key words: Ovarian cancer, Ki-67, KRAS, Immunohistochemistry.

Introduction

Ovarian cancer is the deadliest gynecological tumor, which has an approximate 40% overall survival rate for five years after diagnosis.[1-3] According to the World Health Organization (WHO) data published in 2020 ovarian cancer deaths in Bangladesh reached 2,231 or 0.31% of total deaths.[4] Lack of specialized screening methods at an early stage, silent growth, advanced stage of disease at diagnosis and vague symptoms at presentation are major contributing causes to this high death rate.[5]

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Numerous studies have been conducted, and the results have indicated that determining the tumor cells' proliferative activity would aid in diagnosis and prognosis.[6,7] The clinical behavior and aggressiveness of ovarian carcinoma are significantly influenced by cell division. This non-histone nuclear protein Ki-67 is expressed in all active phases of cell cycle.[7] The nuclear antigen Ki-67 can be stained with an immunohistochemical method to measure the proliferation of cancer cells. The prognostic role of Ki-67 was explored in a broad spectrum of cancer types.[6] Research found strong correlation of Ki-67 protein with malignancies; therefore, the protein is suggested to be a marker of cancer proliferation.[8] Ki-67 is clinically significant for determining tumor aggressiveness and metastasis. Determination of expression of Ki-67 is a technique that is both cost-effective and reliable for

assessing the proliferative index of neoplastic lesions.[9] Ovarian tumors exhibit heterogeneity and differ in the extent to which the disease progresses. Ki-67 expression marker is preferred than other immunohistochemical markers like estrogen receptors, progesterone receptors and p53 gene because of its strong correlation with the ovarian cancer histological stage and grade.[10] Correlation between poor prognosis of ovarian cancer and high levels of Ki-67 expression is seen in several studies which impact on ovarian cancer patients' survival. Platinum resistance and decrease survival are associated with low expression of Ki-67 in high grade serous ovarian cancer.[11]

KRAS (Kristen Rat Sarcoma Virus) protein is a member of the RAS (Rat sarcoma virus) superfamily RAS-like GTPases. KRAS protein is produced by the *KRAS* gene and helps with intracellular signal transduction. *KRAS* is a tumor suppressor gene when it is not altered, but it can turn oncogenic when mutation occurs. KRAS activation leads to downstream signaling of different pathway which promotes cellular proliferation, differentiation and resist apoptosis.[12] In order to control a number of physiological and pathological traits in ovarian cancer, RAS has been shown to interact with numerous additional tumour-inducing and tumour-suppressive pathways. Cisplatin resistance in ovarian cancer is shown to be regulated by mutant RAS interaction with the tumour suppressor gene p53. Mutation of this *KRAS* gene leads to abnormal protein expression and plays role in malignancies by triggering uncontrolled cell proliferation.[3] KRAS is the most frequent abnormal protein which express in ovarian cancer.[3,13]

Materials and Methods

Cross-sectional analytical study was done in the Department of Anatomy of the Bangladesh Medical University (BMU) from March 2023 to February 2024. After getting ethical approval from BMU and National Institute of Cancer Research Hospital (NIRCH), 40 ovarian cancer patients were contacted based on hospital records from the Department of Histopathology, NIRCH. Among them some were excluded due to missing tissue blocks or insufficient cancer tissue in a block. Finally, 30 formalin-fixed paraffin-embedded (FFPE) ovarian cancer blocks were selected. Reproductive and cancer related characteristics of the ovarian cancer patients were collected from the patients, their relatives and from hospital and medical reports after obtaining informed written consent. A total of 20 FFPE noncancerous ovarian tissue blocks were also collected from the above-mentioned institute by the same procedure. Laboratory work was done at the Department of Histopathology, NICRH.

FFPE tissue blocks were cut into 3µm thick sections and incubated in an incubator at 37°C temperature and deparaffinization was done in a hot air oven at 60-65°C for 30 minutes. Then, clearing was done in xylene (three changes) and rehydrated gradually in decreasing concentrations (100%, 90%, 80%, 70%) of isopropyl alcohol. The slides were treated with Dako target retrieval solution in Dako Omnis Autostainer (Dako, Denmark, Glostrup) and rinsed with Tris buffer solution (TBS; pH 7.4). Then, the slides were incubated in 100-150µL PRB (peroxidase blocking reagent) to block the endogenous peroxidase activity. Then the slides were incubated for 20 min with primary antibodies Ki-67 (FLEX monoclonal Mo A Hu Ki-67 Antigen, MIB-1, Dako, Denmark) for assessing Ki-67 expression and KRAS antibody (K-ras Monoclonal Antibody, 9.13, Thermo Fisher, USA) for assessing KRAS expression. Then, the slides were washed in TBS and incubated in horseradish peroxidase blocking reagent for 30 minutes. After that, staining and counter-staining were done with diaminobenzidine and hematoxylin, respectively. Positive controls (vermiform appendix for Ki-67 and colon cancer tissue for KRAS protein) were stained as described for tumor specimens.

The slides were scored and validated by a Histopathologist. Ki-67 positive cells were identified on the basis of brown stained nuclei. The counting was done either by identifying hot spots or by global method.[14] In hot spot method, four hot spots of Ki-67 positive cells (if clearly seen) were identified for assessing the expression of Ki-67. At least 100 nuclei in each field were counted and total 500 nuclei were counted from the four hot spots. If the hot spots were unavailable in the slides, then the global method was followed (one field with highest Ki-67 index, containing 500 nuclei). The Ki-67 index was calculated by the total number of Ki-67 positive nuclei divided by total number of counted cancer cells under high power magnification (×400). Finally, Ki-67 index was expressed in percentage. Ki-67 expression was divided into two separate groups, <20% consider as Ki-67 low expression and ≥20% high expression of Ki-67. According to Rödel et al (2020) ≤20% consider

as Ki-67 low expression and >20% high expression of Ki-67 (Figure 1).[15]

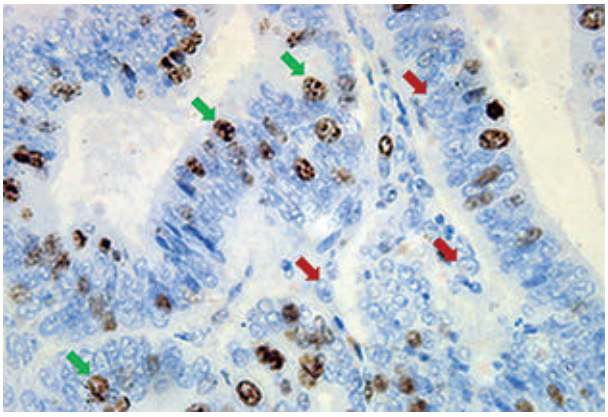

Figure 1: Photomicrograph of positive expression (indicate by green arrows) and negative expression (indicate by red arrows) of Ki-67 (at ×400 magnification).

KRAS positivity was defined as brown cytoplasmic staining of tumour cells. The scoring for KRAS was calculated as follows: in each field, the total number of cells and the number of KRAS positive cells were counted, the average percentage of KRAS positive cells was detected, and the percentages were divided into four grades and recorded as 0, 1, 2, or 3.[16] A semiquantitative score ranging from negative 0 (no staining or, ≤10% of cells stained) to 3+ (1+ staining in 11-30% of the cells: weak, 2+ staining in 31-50% of cells: moderate, and 3+ staining in >50% of cells: intense) was used (Figure 2).[12]

Statistical analysis was done using SPSS, version 27.0. Expression of Ki-67 and KRAS in ovarian cancer tissue was compared with that of non-cancerous ovarian tissue using Fisher's Exact test. Fisher's Exact test was also done to analyze the association between the expressions of Ki-67 and KRAS in ovarian cancer tissue. Association of Ki-67 and KRAS with the clinicopathological characteristics of ovarian cancer patients were also performed using Chi-square (χ^2) test and Fisher's Exact test. In all cases *p* value < 0.05 was considered as statistically significant.

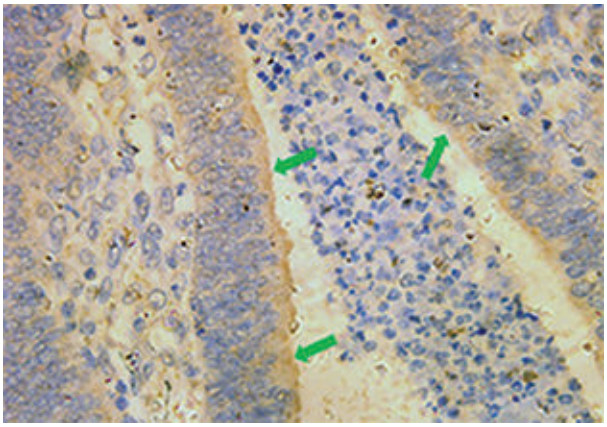

Figure 2: Photomicrograph of positive expression (indicate by green arrows) of KRAS (at ×400 magnification).

Results

The expression of Ki-67 was significantly high in cancerous ovarian tissue than non-cancerous ovarian tissue (Table I). High expression (Ki-67 index e"20%) of Ki-67 was found 60% of ovarian cancer tissue, the mean difference between the high and low expression of Ki-67 was found high statistically significant and *p*< 0.001 (Table II).

Table I: Expression status of Ki-67 in ovarian cancer (n = 30) and non-cancerous (n = 20) ovarian tissue

Ki-67 expression	Cancerous Tissue (n = 30) n (%)	Non-Cancerous Tissue (n = 20) n (%)	*p*
High	18 (60)	0 (0)	<0.0001
Low	12 (40)	20 (100)	

Fisher's Exact Test was done and p < 0.0001 was considered as Significant

Table II: Expression status of Ki-67 in ovarian cancer tissue (n = 30)

Ki-67 expression status	n (%)	Mean ± SD	*p*
High (Ki-67 index ≥20%)	18 (60)	34.39 (12.33)	<0.001
Low (ki-67 index < 20%)	12 (40)	5.75 (4.94)	

Unpaired t test was done, *p*<0.05 was considered as significant

KRAS was expressed in 20% of ovarian cancer tissue, whereas, none of the non-cancerous ovarian tissue expressed the protein (Table III). Significant association was found between the expressions of Ki-67 and KRAS proteins in ovarian cancer tissue. All KRAS-positive ovarian cancer tissues also exhibit high Ki-67 expression (Table IV).

Most of the cancers were epithelial (26 out of 30) and serous (20/26) in histological type. More than 73% of the epithelial ovarian cancers were diagnosed as histological grade III. Peritoneal metastasis was found among 43.3% of the ovarian cancer patients and pelvic organs and lymph nodes involvement was found in 30% of patients. The KRAS expression was significantly associated with pelvic organs and lymph nodes involvement by the cancer (Table V).

Table III: Expression of KRAS in cancerous (n = 30) and non-cancerous (n = 20) ovarian tissue

KRAS expression	Cancer tissue (n = 30) n (%)	Non-cancer tissue (n = 20) n (%)	*p*
Positive	6 (20)	0	0.037
Negative	24 (80)	20 (100)	

Fisher's Exact test was done, $p<0.05$ was considered as significant

Table IV: Association between the expression of Ki-67 and KRAS in ovarian cancer tissue (n = 30)

Expression of KRAS	Expression of Ki-67 High	Low	Total	*p*
Positive	6	0	6	
Negative	12	12	24	0.031
Total	18	12	30	

Fisher's Exact test was done, $p<0.05$ was considered as significant

Table V: Expression of Ki-67 and KRAS with clinicopathological characteristics of ovarian cancer patients (n = 30)

Clinicopathological characteristic	Expression of Ki-67 High (n =18) n (%)	Low (n=12) n (%)	*P*	Expression of KRAS Positive (n= 6) n (%)	Negative (n= 24) n (%)	*P*
Histological types						
Epithelial	15 (83.33)	11 (91.67)	0.73[a]	5 (83.33)	21 (87.50)	0.60 [a]
Serous	12	8		5	15	
Mucinous	1	2			3	
Endometriod	2	1			3	
Germ cell tumor	3 (16.67)	1 (8.33)		1 (16.67)	3 (12.50)	
Histological grades						
Non-graded	2 (11.11)	1 (8.33)	0.36[a]	1 (16.67)	2 (8.33)	0.64[a]
Grade I	0	2 (16.67)		0	2 (8.33)	
Grade II	2 (11.11)	1 (8.33)		0	3 (12.51)	
Grade III	14 (77.78)	8 (66.67)		5 (83.33)	17 (70.83)	
Peritoneal metastasis						
Present	9 (50)	4 (33.33)		4 (66.67)	9 (37.50)	
Absent	9 (50)	8 (66.67)	0.30[b]	2 (33.33)	15 (62.50)	0.20[b]
Pelvic organs and lymph nodes metastasis						
Present	7 (38.89)	2 (16.67)		4 (66.67)	5 (20.83)	
Absent	11 (61.11)	10 (83.33)	0.19[b]	2 (33.33)	19 (79.17)	0.04[b]

n, number; [a]Chi-square (χ^2) or [b]Fisher's Exact test was done; $p<0.05$ considered as significant.

Discussion

Proliferating action of the ovarian cancer cells has been analysed immunohistochemically using antibodies that identify the nuclear antigen Ki-67, which is expressed in proliferating cells.[5] The expression index of the proliferative marker Ki-67 may serve as a useful independent prognostic factor and an indicator of the aggressiveness of serous ovarian tumor.[11] Specifically in proliferating cells, inactivation of the proliferation marker Ki-67 will result in cell death; as a result, it may be a viable indicator of treatment option for ovarian cancer as well as many other cancers.[17]

In this present study the mean difference of the expression of Ki-67 in ovarian cancerous tissue was found significantly high than that of the non-cancerous ovarian tissue. In an Indian study the expression of Ki-67 was found statistically significant between the benign and malignant ovarian tumors.[9] The mean difference of the high and low expression of Ki-67 in ovarian cancerous tissue was found statistically significant in this study. In this present study 60% (12 out of 20) of the serous tumors and 63% (14 out of 22) of grade III tumors were in high Ki-67 expression group, though the association of K-67 expression with tumor type and histological grade was not statistically significant. Statistically significant association of Ki-67 expression with the histological type and grade ovarian cancer was found in an Indian study.[10] In a Chinese study, statistically significant association was observed between Ki-67 expression and age at diagnosis and histological grades of ovarian cancer.[8] We did not find any association with tumor type and grade probably due to small number of other types and grades of ovarian cancers than serous and grade III.

KRAS protein is independent and statistically significant prognostic factor for ovarian cancer patient outcomes.[18] The *KRAS* mutation, one of the most common mutations in ovarian cancer, is thought to be a biomarker for chemoresistance and poor clinical outcomes.[3] Cytoplasmic expression of KRAS protein is higher in ovarian cancer patients seen in some studies. Dysregulation seen in ovarian cancer due to frequent disruption of the EGFR (*Epidermal growth factor receptor)*- signaling pathway, which could be explained due to KRAS overexpression. This correlation between the ovarian cancer mechanism and KRAS expression could help in designing potential genomic targeted therapies for ovarian cancer.[13]

Statistically significant association of the KRAS expression between the ovarian cancer tissue and non-cancerous ovarian tissue was found in this study. In this present study KRAS was expressed in 20% of the ovarian cancer patients. In an Indian study the KRAS expression was found in 47% of ovarian cancer tissue.[19] KRAS expression was found in 53% of ovarian cancer patients in a Chinese study.[20] The expression status of KRAS in above-mentioned studies is higher than this present study may be due to their sample was only epithelial ovarian cancer tissue. Only pelvic organs and lymph nodes involvement was found statistically significant with KRAS expression. This result is consistent with result of an Indian study where the KRAS expression was found statistically significant with metastasis and they also found significant association with histological types which in contrary to this study.[19] Japanese study found that the expression of KRAS was quite common in low grade serous ovarian cancer than the high grade serous ovarian cancer.[21] The finding is contrary with result of this present study because here most of the sample was high grade serous ovarian cancer tissue. This present study found significant association between the expression of Ki-67 and KRAS. Other cancer tissues showed a statistically significant association between the expression of Ki-67 and KRAS.[16,22].

Conclusion

The Ki-67 and KRAS protein expression is significantly high in ovarian cancer tissue and their expression are related to each other. KRAS expression is significantly associated with pelvic organs and lymph nodes involvement in Bangladeshi ovarian cancer patients. Together, these findings indicate that Ki-67 and KRAS may be utilized as reliable biomarkers for predicting the prognosis of epithelial ovarian cancer in the Bangladeshi population. There result also contribute valuable insights to the molecular profiling of ovarian cancer in this population and support the potential of these markers for guiding future diagnostic and prognostic strategies.

Conflict of Interest: There are no conflicts of interest.

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Ethical Clearance: Ethical Clearence was taken from the institutional Review Board IRB Bangladesh Medical University and the IRB of National Institute of Cancer Research and Hospital.

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References

1. Barbero G, Zuntini R, Magini P, Desiderio L, Bonaguro M, Perrone AM, et al. Characterization of BRCA deficiency in ovarian cancer. *Cancers (Basel).* 2023;15:5. DOI:10.3390/cancers15051530.
2. Tang L, Pham NM, Lee AH, Shivappa N, Hébert JR, Zhao J, et al. Dietary inflammatory index and epithelial ovarian cancer in Southern Chinese women: a case-control study. *Cancer Control.* 2020;27:1–9. DOI:s10.1177/1073274820977203.
3. Uddin S, Therachiyil L, Anand A, Azmi A, Bhat A, Korashy HM. Role of RAS signaling in ovarian cancer. *F1000Research.* 2022;11:1–21. DOI:10.12688/f1000research.126337.1.
4. World Health Organization (WHO). World Health Ranking: ovarian cancer. 2020. Accessed August 10, 2025. Available from: https://www.worldlifeexpectancy.com/bangladesh-ovary-cancer.
5. Kasundra K, Jadav K, Patel P, Patel Z. Immunohistochemical study of P53 and Ki-67 expression in surface epithelial tumors of ovary. *Int J Sci Res.* 2023;10(3):43–45. DOI:10.36106/ijsr/0101554.
6. Melati R, Satar MAT, Zakari Z, Hamid A. Ki-67 protein as a prognostic indicator in ovarian carcinoma. *Pertanika J Scholarly Res Rev.* 2018;4:71–77.
7. Staropoli N, Ciliberto D, Iuliano E, Angelis A, Salvino A, Tassone P, et al. Role of P53 and Ki-67 as prognostic factors in ovarian cancer: systematic review and pooled-analysis. *J Cancer Clin.* 2018;1:1002.
8. Wan S, Su C, Wang Y, Ding J, Jiang Q, Ding H, Ni G. Prognostic value of Ki-67 in patients with ovarian cancer. *Eur J Gynaecol Oncol.* 2023;36(11):1502–1507. DOI:10.22514/ejgo.2023.051.
9. Singh N, Kamath SM. Ki-67 immunostaining in epithelial ovarian tumors with clinicopathological correlation. *Int J Med Res Health Sci.* 2022;11:19–26.
10. Verma R, Gupta P, Tiwari N, Lal N, Gupta H, Srivastava A. Histological grade, CA125 levels and IHC expression of ER/PR, HER-2/neu, P53 and Ki-67 markers in epithelial ovarian neoplasms: a correlative study. *Int J Adv Res.* 2017;5:235–254. DOI:10.21474/ijar01/4404.
11. Chen M, Yao S, Cao Q, Xia M, Liu J, He M. The prognostic value of Ki67 in ovarian high-grade serous carcinoma: an 11-year cohort study of Chinese patients. *Oncotarget.* 2017;8:107877–107885. DOI:10.18632/oncotarget.14112.
12. Kanik P, Gajjar K, Ghosh N. Immunohistochemical localization of KRAS and BRAF and its clinical utility in patients with colorectal cancer. *Colorectal Cancer Open Access.* 2018;4. DOI:10.21767/2471-9943.100051.
13. Teasley HE, Beesley A, Kim TH, Risinger J, Young SL, Jeong JW, et al. Differential expression of KRAS and SIRT1 in ovarian cancers with and without endometriosis. *Reprod Sci.* 2020;27:145–151. DOI:10.1007/s43032-019-00017-4.
14. Lashen AG, Toss MS, Ghannam FS, Mokhlouf S, Green A, Mongan NP, et al. The expression, assessment, and significance of Ki-67 expression in breast cancer: an update. *J Clin Pathol.* 2023;76. DOI:10.1136/jcp-2022-208731.
15. Rödel F, Zhou S, Győrffy B, Raab M, Sanhaji M, Mandal R, et al. The prognostic relevance of the proliferation markers Ki-67 and Plk1 in early-stage ovarian cancer patients with serous, low-grade carcinoma based on mRNA and protein expression. *Front Oncol.* 2020;10:558932. DOI:10.3389/fonc.2020.558932.
16. Hanbuli HM El, Sarie MAA. KRAS protein expression in oral squamous cell carcinoma: a potential marker for progression and prognosis. *Iran J Pathol.* 2022;17:469–479.
17. Rahmanzadeh R, Rai P, Celli JP, Rizvi I, Baron-Luhr B, Gerdes J, et al. Ki-67 as a molecular target for therapy in an in vitro 3D model for ovarian cancer. *Cancer Res.* 2010;70(22):9234–9242. DOI:10.1158/0008-5472.CAN-10-1190.
18. Zhang M, Zhuang G, Sun X, Shen Y, Zhao A, Di W. Risk prediction model for epithelial ovarian cancer using molecular markers and clinical characteristics. *J Ovarian Res.* 2015;8:1–12. DOI:10.1186/s13048-015-0195-6.
19. Mandalia T, Dave P, Sinha A, Gajjar K, Ghosh N. Potential role of KRAS and BRAF in epithelial ovarian cancer. *Indian J Gynecol Oncol.* 2021;19. DOI:10.1007/s40944-021-00510-2.
20. Xiao ZH, Linghu H, Liu QF. Expressions of Ras and Sos1 in epithelial ovarian cancer tissues and their clinical significance. *J South Med Univ.* 2016;36:1502–1507. PMID:27881340.
21. Nakayama N, Nakayama K, Yeasmin S, Ishibashi M, Katagiri A, Iida K, et al. KRAS or BRAF mutation status is a useful predictor of sensitivity to MEK inhibition in ovarian cancer. *Br J Cancer.* 2008;99(12):2020–2028. DOI:10.1038/sj.bjc.6604783.
22. Wang J, Dong L, Zheng Z, Zhu Z, Xie B, Xie Y, et al. Effects of different KRAS mutants and Ki67 expression on diagnosis and prognosis in lung adenocarcinoma. *Sci Rep.* 2024:1–11. DOI:10.1038/s41598-023-48307-x.