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IMMUNOHISTOCHEMICAL EXPRESSION OF VIMENTIN IN CERVICAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS

Histopathology

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ABSTRACT

Objective: To evaluate the significance of vimentin expression in cervical carcinoma and correlate it with various prognostic factors like tumor size, tumor type, histological grade, lymph node status and Ki-67 status. **Material and Methods:** The Prospective study was carried out in the Department of Pathology of SRMS IMS, Bareilly over a period of 1.5 years from March 2021 to August 2022.Fifty histologically confirmed cervical carcinoma cases were included. The expression of vimentin and Ki67 proteins in cervical carcinoma tissues were assessed using immunohistochemical method. Correlations between vimentin expression with clinicopathological features and Ki67 status were analyzed . **Results:** Among 50 cases studied, maximum number of patients were diagnosed as squamous cell carcinoma(84%) and rest as adenocarcinoma (16%).Vimentin expression was positive in 28.6% cases of squamous cell carcinoma and 37.5% cases of adenocarcinoma. High Ki67 index was seen in 66.6% cases of SCC and 62.5% cases of adenocarcinoma. No significant correlation was found between vimentin expression and various clinicopathological features like age, tumor size, tumor type, histological grade, lymph node status and clinical stage.However,there was a weak correlation between the expression of vimentin and Ki67status.**Conclusion:** Our findings do not suggest a possible role of Vimentin expression in progression and prognosis of cervical cancer.

KEYWORDS

Squamous cell carcinoma(SCC),Adenocarcinoma,Vimentin,Ki67,Cervical carcinoma

INTRODUCTION

Carcinoma of cervix is the fourth most common cancer in women all over the world with an estimated 5,70,000 new cases in 2018[1]. It represents 6.6% of all female cancers. In India, incidence of cervical cancer is 14% among all cancers occurring in women[2] & the latest clinical data show that the incidence of cervical cancer in young women is increasing year by year[3].It remains a leading cause of cancer death inspite of screening and vaccination[4].

The most common histological subtype of cervical cancer is Squamous cell carcinoma(SCC) which accounts for approximately 80% of cases[1]. More than 90% of cervical carcinoma contain DNA sequences of specific Human Papilloma Virus(HPV) types, especially HPV 16 and HPV 18[6].

The second most common tumor type is adenocarcinoma which accounts for 15% cases, and it arises from a precursor lesion called adenocarcinoma in situ. Rarely, Adenosquamous and neuroendocrine carcinoma are found in cervix which account for the remaining 5% of cases[1]

The majority of women with invasive SCC are diagnosed in their mid-40s or 50s;however,cervical carcinoma can occur at almost any age between 17 and 90 years[5].

High-risk HPV are by far the most important factor in the development of cervical cancer[1]. Other risk factors associated with cervical cancer are presence of clinical genital warts, early marriage, repeated childbirth, woman having multiple sexual partners ,increased duration of usage of oral contraceptive pill, which is high in oestrogen, poor genital hygiene, immunodeficiency and smoking[2,5].

Cancer invasion and metastasis are associated with a physiological process, epithelial -mesenchymal transition(EMT)[6,7]. The loss of epithelial phenotype & gaining of mesenchymal properties enable cancer cells to spread to distant organs of the body at a much faster pace.

EMT has been classified into three categories: type I, type II, type III[10,11].Type I occurs during embryogenesis. Type II is associated with the wound healing[9]. Type III is a pathophysiological adaptation of the process and is closely associated with progression of neoplasia occurring in cells containing certain epigenetic and genetic changes[8-11].These changes, notably affect oncogenes and tumor suppressor gene[9].

Vimentin has been described as a canonical biomarker for EMT. It is a type III intermediate filament that is found in the mesenchymal cells of various types of tissues during their developmental stages and that maintains cell and tissue integrity[1]Expression of vimentin helps in evaluating its role in the development and progression of human cancers

Various recent studies have demonstrated association of vimentin with cancer invasion and poor prognosis in numerous type of cancers

Expression of the Ki-67 protein (pKi67) is associated with the proliferative activity of intrinsic cell populations in malignant tumors, allowing it to be used as marker of tumor aggressiveness. It is an established prognostic and predictive indicator for the assessment of biopsies from patients with cancer.

This study was conducted to detect vimentin expression in cervical carcinoma by immunohistochemical method and to correlate it with clinicopathological factors like tumor size, tumor type, histological grade, lymph node status and Ki-67 status.

MATERIAL AND METHODS

The study was carried out in the Department of Pathology of SRMS IMS, Bareilly over a period of 1.5 years from March 2012 to August 2022.The study population included 50 histopathologically confirmed specimens of cervical carcinoma. These specimens were received in pathology lab from the department of obstetrics and gynecology in 10% formalin. After adequate fixation for 12-24 hours, sections were taken and were submitted for routine processing, followed by paraffin embedding.

3-4 microns thick sections were cut and stained with H&E

(Hematoxylin and Eosin). Two more sections from representative blocks were cut for IHC evaluation, one each for Ki67 and Vimentin markers.

The tumor was classified and graded according to WHO criteria. Various histopathological features like tumor size, tumor type, tumor grade, clinical stage, lymph node status, presence, or absence of lympho-vascular invasion etc were recorded wherever possible.

Case Selection Criteria

Inclusion Criteria

Cervical biopsies and resected hysterectomy specimens histologically proven to be cervical carcinoma.

Exclusion Criteria

- Inadequate tissue
- Poorly fixed/autolyzed tissue
- Non neoplastic cervical lesions

Immunohistochemical Staining Method

Histological sections (3µm thickness) were dewaxed in xylene and rehydrated in graded alcohols. Sections were then washed with water and antigen retrieval was done using pressure cooking, by heating at 110°C, 3 times for 10 minutes each. Slides were then cooled for half an hour. Staining was done in humid chamber using thermoscientific kit and the reagents supplied with the kit. In brief, sections were first washed with TBS and then incubated with 3% hydrogen peroxide to block endogenous peroxidase followed by washing with the supplied buffer. Then Slides were incubated with UltraVisionProtein Block for 10 minutes followed by incubation with primary antibodies (Ki-67 Rabbit Monoclonal Antibody and Vimentin Ab-2) for 1 hour followed by washing with TBS. Incubation with Primary Antibody Amplifier was done for 15 minutes. Slides were again washed with buffer and then incubated with HRP Polymer Quanto for 10 minutes. Subsequently slides were washed with buffer and distilled water. Tissue staining was visualised with a DAB Substrate chromogen solution. Slides were counterstained with haematoxylin, dehydrated, washed, and mounted. Both positive and negative controls were ran simultaneously.

Evaluation Of Vimentin Expression

The expression of vimentin was considered positive if >10% tumor cells showed distinct yellow brown or brown granular cytoplasmic immunoreactivity.

Three highly marked fields were selected and considered for evaluation.

The scoring was based on the percentage of stained cells. It was divided into three groups:

1= 11-40% tumor cells positive
2= 41-75% tumor cells positive
3= ≥76% tumor cells positive

Evaluation Of Ki-67 Expression

Positive Ki-67 is defined as the presence of yellow brown or brown nuclear staining in >10% tumor cells.

The sections stained for Ki-67 proliferation index were evaluated using scores from 1 to 3.

1= low proliferation, that is 10-15% positive cells
2= intermediate proliferation, that is 16-30% positive cells
3= high proliferation, that is more than 30% positive cells

Statistical Analysis

Relationship between Ki-67,Vimentin expression and the patients clinicopathological features were then analyzed in MS Excel. P values were calculated using Fisher's Exact test and T test (95% confidence interval for significance; tool used: data analysis in MS Excel). Chi Square Values calculated using Chi test (Tool used: MS Excel).

RESULTS

In the present study a total of 50 cases were studied with the patient age ranged from 51-60 years with a mean age of 55 years.

Most common presenting complaint was bleeding per vaginum (36%) followed by postmenopausal bleeding (30%) .

Maximum number of patients were diagnosed as SCC(84%) and rest as Adenocarcinoma(16%).

Thirty-nine cases(92.8%) of SCC cases were graded as moderately differentiated, one(2.4%) as well differentiated and two(4.8%) as poorly differentiated.

Two cases of adenocarcinoma were graded as poorly differentiated. Moderately and well differentiated cases were three (37.5%) each.

Vimentin expression in SCC was positive in 28.6% cases and negative in 71.4% cases while Vimentin expression in Adenocarcinoma was positive in 37.5% cases and negative in 62.5% cases.

Vimentin scores in SCC were Score 1(12% cases),Score 2 (9.5 % cases) and Score 3 (7.1% cases) whereas Vimentin scores in adenocarcinoma were Score 1(25% cases),Score 2 (12.5% cases) and Score 3(12.5% cases).

Thirty-seven(88%) cases showed positive Ki67 expression in SCC and 7(87.5%) cases showed positive Ki67 expression in Adenocarcinoma. High Ki67 index (score 3) was seen in most of the cases[SCC(66.6%) and Adenocarcinoma(62.5%) cases.

Most common clinical stage was stage IIB in SCC while Clinical staging was available only in two cases of adenocarcinoma with one case of stage IIB and other of stage IIIB.

There was statistically no significant correlation between vimentin expression and various clinicopathological features like age, tumor size, tumor type, histological grade, lymph node status and clinical stage.

There was a weak correlation between the expression of vimentin and Ki67.

Table 1. Expression Of Vimentin With Clinicopathological Features

S.No.	Clinicopathological Feature	No. of cases	Vimentin		P value
			Negative	Positive	
1	Age 31—50 years	17	12(71%)	5(29%)	0.948*
	51—70 years	31	23(74%)	8(26%)	
	71—80 years	2	0(0%)	2(100%)	
2	Tumor size ≤ 5.5cm	30	23(77%)	7(23%)	0.528*
	□ 5.5cm	20	13(65%)	7(35%)	
3	Tumor type SCC	42	30(71%)	12(29%)	0.613*
	Adenocarcinoma	8	5(63%)	3(8%)	
4	Tumor grade in SCC WD	1	1(100%)	0(0%)	0.524*
	MD	39	27(69%)	12(31%)	
	PD	2	2(100%)	0(0%)	
5	Tumor grade in adenocarcinoma WD	3	2(67%)	1(33%)	0.714**
	MD	3	2(67%)	1(33%)	
	PD	2	1(50%)	1(50%)	
6	FIGO Stage in SCC IB3	1	1(100%)	0(0%)	0.489*
	IIA	1	1(100%)	0(0%)	
	IIA2	5	2(40%)	3(60%)	
	IIB	9	8(89%)	1(11%)	
	IIIA	4	3(75%)	1(25%)	
	IIIB	5	5(100%)	0(0%)	
	IIIC	1	0(0%)	1(100%)	
	IVA	2	2(100%)	0	
	IVB	1	1(100%)	0	
7.	FIGO Staging in Adenocarcinoma IIIB	1	1(100%)	0	NA
	IIB	1	1(100%)	0	

WD-Well differentiated, MD-Moderately differentiated, PD-Poorly differentiated

*Pearson's chi-squared test, **Fisher's exact test

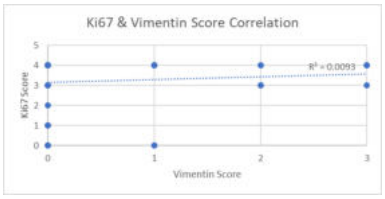

Figure 1. Correlation Between Vimentin And Ki-67 Expression

Figure 1 shows a weak correlation between Vimentin expression and Ki67.(R^2=0.0093)

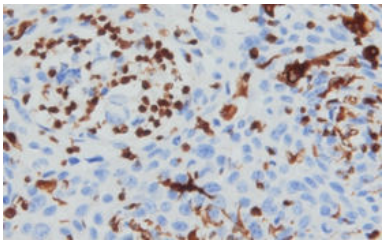

Figure 2.vimentin Expression (score 1) 40x

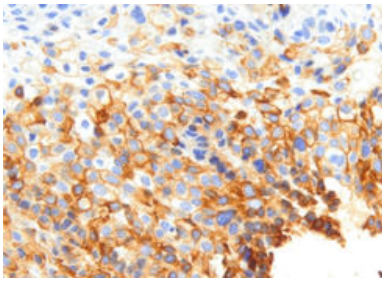

Figure 3.vimentin Expression (score 2) 40x

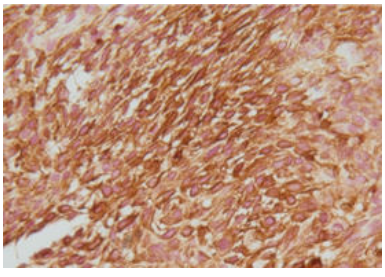

Figure 4.vimentin Expression (score 3) 40x

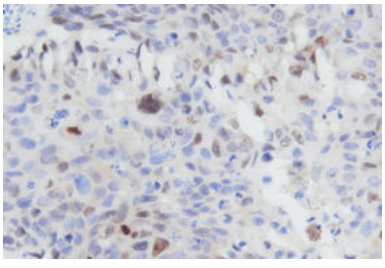

Figure 5.ki-67 Expression (score 1) 40x

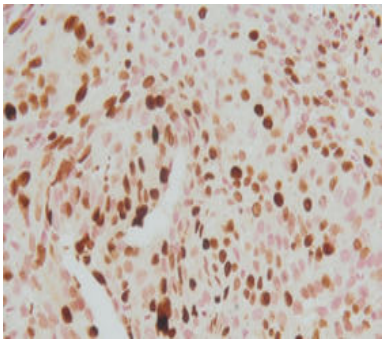

Figure 6.ki-67 Expression (score 2) 40x

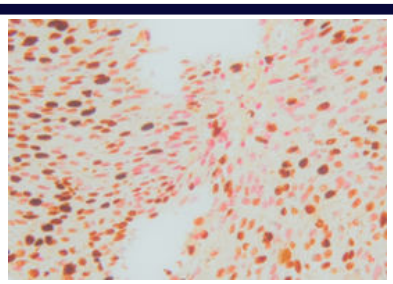

Figure 7.ki-67 Expression (score 3) 40x

DISCUSSION

Cervical cancer is the fourth most common cancer after breast, colorectal, and lung cancer in women. According to GLOBOCAN 2012 statistics, there were an estimated 266,000 deaths from cervical cancer worldwide in 2012, accounting for 7.5% of all female cancer deaths.[12]

Even with best available therapies, many patients die due to metastasis or other consequences of cervical cancer.[13]Exact reason of development and progression of cervical carcinoma is not well known. The hyperexpression of vimentin during the EMT event seems to promote greater stabilization and a greater cellular migration, increasing the invasive capacity of the cells, this characteristic leads to a worse prognosis for tumors(Phua et al.[14] ,Satelli and li.[15]).

The unusual expression of vimentin during reactivation of the EMT program in the invasion and metastasis process has already been investigated in several human tumors like bladder cancer[16] ,vulvar carcinoma[17] ,prostate cancer[18]and colorectal cancer[19].
In cervical cancer, only few studies have been done so far to evaluate the association of vimentin expression with prognostic aspects of tumors.

Assessment of Ki-67 protein expression by IHC is regarded as reliable method to reflect the activity of cell proliferation. A lot of studies displayed that Ki-67 was highly expressed in a variety of gynecological malignant tumors such as ovarian cancer, endometrial cancer, and breast cancer , and was related to tumor occurrence, progression, invasion, and metastasis intimately(Kilickap[20] et al.,Kucukyoz[21] et al.,Shevra[22] et al.).

Combined expression of Vimentin and Ki-67 in the postoperative pathological diagnosis is of great importance for judging the histological behavior of cervical carcinoma patient prognosis[23].

In the present study, maximum patients were in the age group of 51 to 60 years, with a mean age of 55 years. S.Aswathy et al.[24].Wright et al[25] ,Crammeret al[26] ,Raoet al[27], Wahiet al[28] ,L.Jiaying et al[29]and RK Naveen et al found similar distribution in their studies.

The most common presenting symptom of cervical cancer in our study was bleeding per vaginum. Post-menopausal bleeding was observed as the next common presenting feature. S.Madhutandra et al[30]revealed similar result.SL van Schalkwyk et al[31]found offensive vaginal discharge as most common symptom and AD Mwaka et al[33] found intermenstrual bleeding as common presenting complaint.

Majority of the cases(84%) in our study were squamous cell carcinoma. Only 8 cases(16%) of adenocarcinoma cervix were noted. This is in accordance with the findings of Boschet al[32], W.Meget al[34] , FC Santana et al.[35] and B.Sine et al.[36] who got similar results.

Cases of adenocarcinoma have also increased over time in relative distribution as compared to squamous cell carcinoma in developed countries. Possible reason includes increased prevalence of adenocarcinoma risk factors such as obesity, nulliparity or HPV 18[37].

In our study most of the cases of SCC were graded as moderately differentiated. MDC Young et al[38] and E.Ancuta et al[39]also showed similar results, whereas RK Naveen et al , E.Nazik et al[40] ,Y.Jian-qin et al[23],CR Hunt et al [41] and Suo et al[42] found most of the cases to be poorly differentiated.

In the present study the cases of Adenocarcinoma were almost equally distributed among the three grades. This could be due to only few

number of cases of adenocarcinoma.

In present study vimentin expression was present in only 28.6% of the SCC cases. Similar result was shown by L.Jiaying et al[6], F.C Santana et al[35]and C R Hunt et al[41].,whereas MDC Yong et al[38] Y.Jian-qin et al[23] and E.Nazik et al[40] found vimentin to be positive in higher number of cases constituting 87%, 75% and 40% of cases respectively.

Expression of vimentin in cases diagnosed as adenocarcinoma was negative in 62.5% cases. Similar result was shown by F.C Santana et al[35](77%),C.R Hunt et al[41](80%) and L.Jiaying[6](75%).

Present study revealed positive expression of Ki67 in 88% cases of squamous cell carcinoma and 87.5% cases of adenocarcinoma. This was in concordance with the studies conducted by Y.Jian-qin et al[23](100%),H.Jitti et al[43](81.3%),K.Kanjana et al[47](91.3%),S.Qin et al[3] (95.2%)and LI Peng-li et al[4](100%).

In the present study a weak correlation was observed between vimentin expression and Ki67 status whereas Y.Jian-qin et al[23] in their study found a significant positive correlation between vimentin and Ki67 expression. On the other hand, L.Jiaying et al[6] found a negative correlation between vimentin and Ki67 expression.

The difference between these findings can be due to different distribution of grades of the cancer in these studies.

The number of poorly differentiated cases in our study was 8%, in the study done by L.Jiaying et al.[6] ,the cases were 16.9% whereas in the study done by Y.Jian-qin et al[23] ,the poorly differentiated cases were 49.1%.

Possible reason for this weak correlation in our study could be explained in accordance with a study which has reported that cancer stem cells with increased CD44(cell surface adhesion receptor) expression tend to form the negative feedback machinery in terms of oxidative stress-induced Wnt/beta-catenin signal transduction. This negative feedback regulation is exerted by upregulated CD44/Vimentin expression which may be partially responsible for the inversed expression pattern between CD44/Vimentin and Ki67/c-Myc.[6]

Another possible reason could be an observation made by Y Jian-qin et al. in their study that epithelial-mesenchymal transition tends to appear in poorly differentiated cervical squamous cell carcinoma[23].Even RK Naveen et al. also found that the expression of vimentin increased with an increase in the histological grade of the carcinoma cervix.

Clinical stage is a consistent prognostic factor for survival in all cervical carcinoma patients. The survival rates decrease with advancing stage[37].

In the present study clinical staging of tumor was available in 31 cases. Out of which stage IIB was commonest. E.Ancuta et al[39] revealed similar result whereas MDC Yong et al[38], L.Jiaying [6] and Y.Jian-qin et al[23]reported stage I as the most common stage.LI Peng li et al[4]reported equal cases of stage I and II and H.Jitti et al[43] reported stage Ib1 as the commonest stage.

Present study also demonstrated that statistically there was no correlation found between vimentin expression with various clinicopathological features like age, tumor size, tumor type and tumor grade. This is in accordance with the study conducted by F.C Santana et al[35]whereas MDC.Young et al. found the degree of vimentin expression to be significantly correlated with histological grade, nodal metastasis , recurrence and survival in cervical squamous cell carcinoma[38].

L.Jiaying et al[6]. found that vimentin protein expression is strongly associated with the onset age, lymph node metastasis, lymphatic invasion, Ki-67 staining, recurrence, and survival in cervical cancer patients.

Various recent studies have also demonstrated association of vimentin with cancer invasion and poor prognosis in other type of cancers like breast, thyroid, lung, pancreas etc.(MHS Chen et al.[45], M Dauphin et al.[46], H.Jin et al.[47], A Handra-Luca et al.[48], Y.Nami et al.[49], Hemalatha et al.[50], Cristina Mariana et al.[51], S.Yin et al.[52])

This study had several limitations like small number of cases were included. The specimen comprised mainly biopsies and lymph nodes were not submitted. Lymphovascular invasion could not be ascertained in most of the cases and Clinical staging was available in only thirty-one cases.

To conclude, the current findings do not suggest a possible role of Vimentin in progression and prognosis of cervical cancer. However, our study is limited by smaller number of cases and lack of availability of clinical staging in all the cases. As discussed earlier many studies done in tumors like breast, thyroid and pancreas have found immunoexpression of vimentin related to tumor grade and prognosis. But very scanty data is available on role of vimentin in cervical cancer. Hence, more detailed studies are required to establish the role of Vimentin as a prognostic factor in carcinoma cervix.

REFERENCES

1. Kumar, V., & Abbas, A. K. (2017). Aster JC Robbins Basic Pathology E- Book. Amsterdam Nertherlands: Elsevier Health Sciences.
2. Park, K. (2019). Park's Textbook of Preventive & Social Medicine 25th edition Jabalpur. India: M/s Banarasidas Bhanot publishers, 269-280.
3. Shi, Q., Xu, L., Yang, R., Meng, Y., & Qiu, L. (2019). Ki 67 and P16 proteins in cervical cancer and precancerous lesions of young women and the diagnostic value for cervical cancer and precancerous lesions. Oncology letters, 18(2), 1351-1355
4. Li, P. L., & Tan, H. Z. (2015). Expression of PPARγ, p27 and Ki67 in cervical cancer and its clinical significance. J Int Transl Med, 3(1), 11-17
5. Kurman, R. J. (Ed.). (2013). Blaustein's pathology of the female genital tract. Springer Science & Business Media.
6. Lin, J., Lu, J., Wang, C., & Xue, X. (2017). The prognostic values of the expression of Vimentin, TP53, and Podoplanin in patients with cervical cancer. Cancer cell international, 17, 1-12.
7. Brabletz, T., Hlubek, F., Spaderna, S., Schmalhofer, O., Hiendlmeyer, E., Jung, A., & Kirchner, T. (2005). Invasion and metastasis in colorectal cancer: epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and β-catenin. Cells tissues organs, 179(1-2), 56-65.
8. Serrano-Gomez, S. J., Maziveyi, M., & Alahari, S. K. (2016). Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. Molecular cancer, 15(1), 1-14.
9. Kalluri, R., & Weinberg, R. A. (2010). The basics of epithelial-mesenchymal transition. The Journal of clinical investigation, 120(5), 1786-1786.
10. Nieto, M. A. (2013). Epithelial plasticity: a common theme in embryonic and cancer cells. Science, 342(6159), 1234850
11. Bill, R., & Christofori, G. (2015). The relevance of EMT in breast cancer metastasis: Correlation or causality?. FEBS letters, 589(14), 1577-1587.
12. Pan, D., Wei, K., Ling, Y., Su, S., Zhu, M., & Chen, G. (2015). The prognostic role of Ki-67/MIB-1 in cervical cancer: a systematic review with meta-analysis. Medical science monitor: international medical journal of experimental and clinical research, 21, 882.
13. Piri, R., Ghaffari, A., Gholami, N., Azami-Aghdash, S., PourAli-Akbar, Y., Saleh, P., & Naghavi-Behzad, M. (2015). Ki-67/MIB-1 as a prognostic marker in cervical cancer-a systematic review with meta-analysis. Asian Pacific Journal of Cancer Prevention, 16(16), 6997-7002.
14. Phua, D. C., Humbert, P. O., & Hunziker, W. (2009). Vimentin regulates scribble activity by protecting it from proteasomal degradation. Molecular biology of the cell, 20(12), 2841-2855.
15. Satelli, A., & Li, S. (2011). Vimentin in cancer and its potential as a molecular target for cancer therapy. Cellular and molecular life sciences, 68, 3033-3046.
16. Zhao, J., Dong, D., Sun, L., Zhang, G., & Sun, L. (2014). Prognostic significance of the epithelial-to-mesenchymal transition markers e-cadherin, vimentin and twist in bladder cancer. International braz j urol, 40, 179-189.
17. Holthoff, E. R., Spencer, H., Kelly, T., Post, S. R., & Quick, C. M. (2016). Pathologic features of aggressive vulvar carcinoma are associated with epithelial-mesenchymal transition. Human pathology, 56, 22-30.
18. Figiel, S., Vasseur, C., Bruyere, F., Rozet, F., Maheo, K., & Fromont, G. (2017). Clinical significance of epithelial-mesenchymal transition markers in prostate cancer. Human pathology, 61, 26-32.
19. Du, L., Li, J., Lei, L., He, H., Chen, E., Dong, J., & Yang, J. (2018). High vimentin expression predicts a poor prognosis and progression in colorectal cancer: a study with meta-analysis and TCGA database. BioMed Research International, 2018.
20. Kilickap, S., Kaya, Y., Yucel, B., Tuncer, E., Babacan, N. A., & Elagoz, S. (2014). Higher Ki67 expression is associates with unfavorable prognostic factors and shorter survival in breast cancer. Asian Pacific Journal of Cancer Prevention, 15(3), 1381-1385.
21. Kucukgoz Gulec, U., Gumurdulu, D., Guzel, A. B., Paydas, S., Seydaoglu, G., Acikalin, A., ... & Altintas, A. (2014). Prognostic importance of survivin, Ki-67, and topoisomerase IIα in ovarian carcinoma. Archives of gynecology and obstetrics, 289, 393-398.
22. Shevra, C. R., Ghosh, A., & Kumar, M. (2015). Cyclin D1 and Ki-67 expression in normal, hyperplastic and neoplastic endometrium. Journal of postgraduate medicine, 61(1), 15.
23. Yu, J. Q., Zhou, Q., Zheng, Y. F., & Bao, Y. (2015). Expression of vimentin and Ki-67 proteins in cervical squamous cell carcinoma and their relationships with clinicopathological features. Asian Pacific Journal of Cancer Prevention, 16(10), 4271-4275.
24. Sreedevi, A., Javed, R., & Dinesh, A. (2015). Epidemiology of cervical cancer with special focus on India. International journal of women's health, 405-414.
25. Wright Jr, T. C. (2006). CHAPTER 3 Pathology of HPV infection at the cytologic and histologic levels: Basis for a 2□tiered morphologic classification system. International Journal of Gynecology & Obstetrics, 94, S22-S31.
26. Cramer, D. W. (1974). The role of cervical cytology in the declining morbidity and mortality of cervical cancer. Cancer, 34(6), 2018-2027.
27. PS, R., RS, R., & DJ, R. (1959). A study of the aetiological factors in carcinoma of cervix uteri in Guntur. Journal of the Indian Medical Association, 32(12), 463-470.
28. Wahi, P. N., Mali, S., & Luthra, U. K. (1969). Factors influencing cancer of the uterine cervix in North India. Cancer, 23(5), 1221-1226.
29. Lin, J., Lu, J., Wang, C., & Xue, X. (2017). The prognostic values of the expression of Vimentin, TP53, and Podoplanin in patients with cervical cancer. Cancer cell international, 17, 1-12.

30. Sarkar, M., Konar, H., & Raut, D. K. (2010). Symptomatology of gynecological malignancies: experiences in the gynecology out-patient clinic of a tertiary care hospital in Kolkata, India. Asian Pac J Cancer Prev, 11(3), 785-91.
31. van Schalkwyk, S. L., Maree, J. E., & Dreyer Wright, S. C. (2008). Cervical cancer: the route from signs and symptoms to treatment in South Africa. Reproductive health matters, 16(32), 9-17.
32. Bosch, F. X., & De Sanjosé, S. (2003). Chapter 1: Human papillomavirus and cervical cancer—burden and assessment of causality. JNCI monographs, 2003(31), 3-13.
33. Mwaka, A. D., Orach, C. G., Were, E. M., Lyratzopoulos, G., Wabinga, H., & Roland, M. (2016). Awareness of cervical cancer risk factors and symptoms: cross□sectional community survey in post□conflict northern Uganda. Health Expectations, 19(4), 854-867.
34. Watson, M., Saraiya, M., Benard, V., Coughlin, S. S., Flowers, L., Cokkinides, V., ... & Giuliano, A. (2008). Burden of cervical cancer in the United States, 1998–2003. Cancer, 113(S10), 2855-2864.
35. Santana, F. C., Ramos, J. E. P., Nogueria, N. A., Líbera, L. S. D., Aparecida, T., Rabelo, S. H., & Saddi, V. A. Vimentin is not a reliable prognostic biomarker for cervical cancer.
36. Bayo, S., Bosch, F. X., de Sanjosé, S., Muñoz, N., Combita, A. L., Coursaget, P., ... & Meijer, C. J. (2002). Risk factors of invasive cervical cancer in Mali. International journal of epidemiology, 31(1), 202-209.
37. Gien, L. T., Beauchemin, M. C., & Thomas, G. (2010). Adenocarcinoma: a unique cervical cancer. Gynecologic oncology, 116(1), 140-146.
38. Cheng, Y., Zhou, Y., Jiang, W., Yang, X., Zhu, J., Feng, D & Ling, B. (2012). Significance of E-cadherin, β-catenin, and vimentin expression as postoperative prognosis indicators in cervical squamous cell carcinoma. Human pathology, 43(8), 1213-1220.
39. Ancuta, E., Ancuta, C., Cozma, L. G., Iordache, C., Anghelache-Lupascu, I., Anton, E. & Chirieac, R. (2009). Tumor biomarkers in cervical cancer: focus on Ki-67 proliferation factor and E-cadherin expression. Rom J Morphol Embryol, 50(3), 413-8.
40. Husain, N. E. O., Babiker, A. Y., Albutti, A. S., Alsahli, M. A., Aly, S. M., & Rahmani, A. H. (2016). Clinicopathological significance of vimentin and cytokeratin protein in the genesis of squamous cell carcinoma of cervix. Obstetrics and gynecology international, 2016.
41. Hunt, C. R., Hale, R. J., & Buckley, C. H. (1996). Vimentin intermediate filament expression in uterine cervical carcinoma. International Journal of Gynecological Cancer, 6(5), 376-379.
42. Suo, Z. H. E. N. H. E., Holm, R., & Nesland, J. M. (1992). Squamous cell carcinomas, an immunohistochemical and ultrastructural study. Anticancer research, 12(6B), 2025-2031.
43. Hanprasertpong, J., Tungsinmunkong, K., Chichareon, S., Wootipoom, V., Geater, A., Buhachat, R., & Boonyapipat, S. (2010). Correlation of p53 and Ki□67 (MIB□1) expressions with clinicopathological features and prognosis of early stage cervical squamous cell carcinomas. Journal of Obstetrics and Gynaecology Research, 36(3), 572-580.
44. Kanthiya, K., Khunnarong, J., Tangjitgamol, S., Puripat, N., & Tanvanich, S. (2016). Expression of the p16 and Ki67 in cervical squamous intraepithelial lesions and cancer. Asian Pacific Journal of Cancer Prevention, 17(7), 3201-3206.
45. Chen, M. H. S., Wai-Cheong Yip, G., Tse, G. M. K., Moriya, T., Lui, P. C. W., Zin, M. L., ... & Tan, P. H. (2008). Expression of basal keratins and vimentin in breast cancers of young women correlates with adverse pathologic parameters. Modern pathology, 21(10), 1183-1191.
46. Dauphin, M., Barbe, C., Lemaire, S., Nawrocki-Raby, B., Lagonotte, E., Delepine, G., ... & Polette, M. (2013). Vimentin expression predicts the occurrence of metastases in non small cell lung carcinomas. Lung cancer, 81(1), 117-122.
47. Yin, S., Chen, F. F., & Yang, G. F. (2018). Vimentin immunohistochemical expression as a prognostic factor in gastric cancer: A meta-analysis. Pathology-Research and Practice, 214(9), 1376-1380.
48. Handra-Luca, A., Hong, S. M., Walter, K., Wolfgang, C., Hruban, R., & Goggins, M. (2011). Tumour epithelial vimentin expression and outcome of pancreatic ductal adenocarcinomas. British journal of cancer, 104(8), 1296-1302.
49. Yamashita, N., Tokunaga, E., Kitao, H., Hisamatsu, Y., Taketani, K., Akiyoshi, S., ... & Maehara, Y. (2013). Vimentin as a poor prognostic factor for triple-negative breast cancer. Journal of cancer research and clinical oncology, 139, 739-746.
50. Hemalatha, A., Suresh, T. N., & Kumar, M. H. (2013). Expression of vimentin in breast carcinoma, its correlation with Ki67 and other histopathological parameters. Indian journal of cancer, 50(3), 189-194.
51. Calangiu, C. M., Simionescu, C. E., Stepan, A. E., Cernea, D., Zăvoi, R. E., & Mărgăritescu, C. L. A. U. D. I. U. (2014). The expression of CK19, vimentin and E-cadherin in differentiated thyroid carcinomas. Rom J Morphol Embryol, 55(3), 919-25.
52. Jin, H., Morohashi, S., Sato, F., Kudo, Y., Akasaka, H., Tsutsumi, S., ... & Kijima, H. (2010). Vimentin expression of esophageal squamous cell carcinoma and its aggressive potential for lymph node metastasis. Biomedical Research, 31(2), 105-112.