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ROLE OF p16 AND Ki67 BIOMARKERS IN CERVICAL CANCER: A REVIEW

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Abstract:

Cervical cancer is one of the most common and dangerous diseases in India and even globally which accounts for around 5% deaths per annum. Screening of cervical cancer is the only step which is proven to be most significant in prevention from high mortality rates. There are various types of techniques developed till date like liquid based cytology, HPV DNA testing and Pap tests which are helpful in screening of cervical cancer, but such techniques are associated with some kind of limitations related to sensitivity and specificity. It leads to the need of some novel technique which can provide results with high sensitivity and specificity. In the research for the same, there are many biomarkers which are developed till date. In this literature review, we will study characteristic features of p16 which acts as a cell cycle regulatory protein that has the ability to induce cell cycle arrest and one other Ki-67 which acts as cell proliferation marker. They could not co-express in the same cervical epithelial cells under physiological conditions. The co-expression of these two molecules shows that HR-HPV inflammation causes a disruption of the cell cycle and indicates the existence of high-grade cervical epithelial lesions. The rates of p16 and Ki67 expression were shown to be closely related to the occurrence of cervical lesions. In this review, along with characteristics of p16 and Ki-67, we will study the significance of p16 and Ki-67 dual-staining technique which showed some good results in efficacy of screening of cervical cancer.

Key Words: Cervical cancer; dual-staining technique; Ki67; p16, Screening.

INTRODUCTION:

Cancer is one of the fatal diseases in which cells in the body divide in an uncontrollable manner. After being cancerous, the cell cannot function as a normal cell. Cancer has the potential to grow anywhere in the body and can spread to any part of the body. Cancerous cells lack the components that

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instruct them to stop dividing and to die. There can be many ways that can cause cancer in humans, in which some are preventable also. Some of such methods are heavy alcohol consumption, excess body weight, physical inactivity and poor nutrition. Sometimes, cancer can be through gene--tic reasons also. A person's genetic code tells their cells when to divide and expire. Changes in the genes can lead to faulty instructions, and cancer can result. Genes also influence the cells' production of proteins, and proteins carry many of the instructions for cellular growth and division. Some genes change proteins that would usually repair damaged cells. This can lead to cancer[1]. Risk of infection with cancer increases with increasing age because the body's ability to eliminate cells with damaged DNA before they turn cancerous goes down as we age.

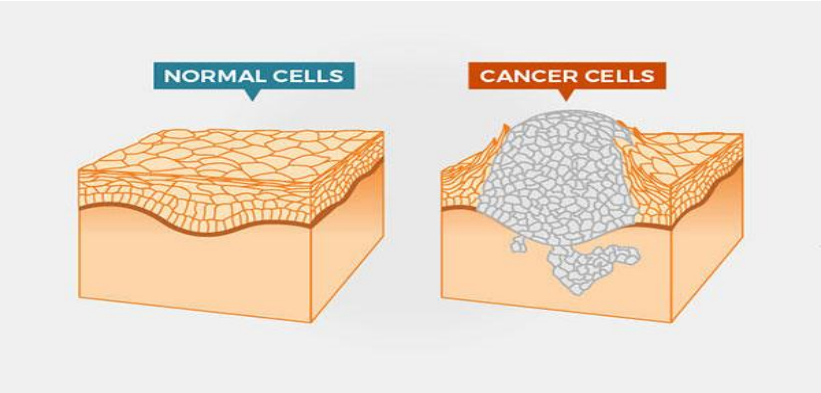

Fig 1: Structural difference between normal cell and cancer cell. [2]

Cancer is always named for the part of the body where it starts, even if it spreads to other body parts later. When cancer starts in the cervix, it is called *cervical cancer.* The cervix connects the vagina (birth canal) to the upper part of the uterus. The *uterus* (or womb) is where a baby grows when a woman is pregnant. All women are at risk for cervical cancer. It occurs most often in women over age 30[3]. Conversion of normal cells into cancer cells leads to induction of cervical cancer. It normally takes several years in case of normal immune system to happen but can form in little time also in a weak immune system. On looking globally, Cervical cancer is the fourth most common cancer in women with approximately 570, 000 cases per annum and around 7.5% of all female cancer deaths.In fact, women infected with HIV are more likely to get cervical cancer easily with an estimated rate of 5%.

Role of Human Papillomavirus in Cervical Cancer:

Cervical cancer is one of the most common diseases associated with HPV. However, most HPV infections clear up on their own and most pre-cancerous lesions resolve spontaneously, there is a risk for all women

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that HPV infection may become chronic and pre-cancerous lesions progress to invasive cervical cancer. Lesions generally occur in the squamous intraepithelial region which forms from the abnormal growth of squamous cells on the cervix's surface. Based on how much of the cervix is compromised and how irregular the cells are, the variations in the cells are classified as low grade or high grade.

Early improvements in the composition, shape, and amount of cells that make up the cervix's surface are referred to as low-grade SIL. Low-grade lesions can disappear on their own. Others, on the other hand, can develop larger or irregular over time, creating a high-grade lesion. Low-grade precancerous lesions are also known as mild dysplasia or cervical intraepithelial neoplasia 1 (CIN 1). Early cervix shifts are most common in women between the ages of 25 and 35, although they can also occur in women of all ages.

High-grade SIL indicates the presence of a significant number of precancerous cells that vary from normal cells in appearance. These precancerous modifications, including low-grade SIL, only affect cells on the cervix's surface. For months, if not years, the cells will not become cancerous and infiltrate deeper layers of the cervix. Moderate or extreme dysplasia, CIN 2 or 3, or carcinoma are both terms used to describe high-grade lesions. They are most common in women between the ages of 30 and 40, but they can occur at any age [4].

Two specific types of HPV are included as causative agents for cervical cancer. The squamous cells that line the inner surfaces of these organs are infected by HPV. As a result, the vast majority of HPV-related tumours are squamous cell carcinomas. Adenocarcinomas are cervical tumours that are caused by HPV inflammation of gland cells in the cervix. HPV is quickly transmitted from one sexual partner to the next. It may be spread by vaginal–penile intercourse, penile–anal sex, penile–oral sex, vaginal–oral sex, and the use of sex toys or other items. The virus is readily transmitted from one intimate partner to the next. Condoms and dental dams will reduce the risk of HPV transmission, but they can't absolutely eliminate it [5].

Screening of Cervical Cancer:

Screening is the monitoring of all women who are at risk of cervical cancer, the majority of whom may have no symptoms. Screening is intended to detect precancerous alterations that, if left untreated, will lead to cancer. Screening is only successful where a well-organized protocol for follow-up and care is in place. Women that have anomalies on screening need follow-up, diagnosis, and likely medication.

Cervical screening's main purpose is to identify pre-invasive tumours and prevent cervical cancer mortality by treating precancerous lesions. Screening in developed countries has achieved a decrease in incidence and mortality by about 80%. There are some approaches for screening for cervical cancer precursors which are studied are as follows:

1. PAP Tests: The most used Pap test has a high accuracy but a low sensitivity. A Pap examination extracts cells from the cervix and examines them for anomalies.In developing countries, the Pap test has been effective in lowering cervical cancer mortality rates. Pap examinations, which are highly labour intensive, complex, and time consuming,

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do not diagnose all precancerous lesions. Because of a shortage of resources and well qualified staff to read the smear, they are ineffective in low-resource or emerging health economies [6].

2. HPV DNA Testing: HPV DNA testing is a more modern screening process. According to research, it is more efficient at early detection than a conventional Pap examination since it is more reliable and detects cell defects sooner. Persistent infection of high-risk oncogenic HPV forms, the development of cervical cancer precursor lesions, and the eventual development of cervical cancer are all related. As a result, chronic contamination of oncogenic forms of HPV is linked to the growth of cervical cancer.

   HPV DNA monitoring can be helpful in the triage of low-grade cervical changes, the follow-up of non-confirmed, potential high-grade changes, the post-colposcopy follow-up of CIN 2-3, and the clarification of indeterminate histology [7].

3. Liquid Based Cytology: Liquid-based cytology is an improvement over the traditional PAP Test system. Recent research has revealed that liquid-based cytology can and can be used. However, in terms of sensitivity and accuracy, there hasn't been much evidence of a benefit over traditional Pap tests. As a result, it is also incompatible as a cervical cancer screening tool [8]

These above mentioned methods are less specific or less sensitive like cytology demonstrated high precision ranging from 86 % to 100 %, but poor sensitivity ranging from 30 % to 87 % whereas HPV testing provides high sensitivity upto 95% but it is less specific which convinces females to go for cytology after HPV testing for their own confirmation. As a result, more efficient markers are needed to differentiate HPV-positive women with normal cytology or negative HPV16/18 from cytological ASCUS/LSIL candidates, as well as to distinguish women with possible high-grade CIN from cytological ASCUS/LSIL candidates. A recent study demonstrated that p16/Ki-67 dual-staining cytology is emerging as an alternative biomarker, with overall high sensitivity and specificity for detecting high-grade CIN to reduce referral rates, unnecessary treatments and finally costs.

Characteristics features of p16:

p16 is a protein that acts as a tumour suppressor by slowing cell division by slowing the progression of the cell cycle from the G1 to the S step. It is also known as cyclin-dependent kinase inhibitor 2A, CDKN2A, multiple tumour suppressor 1. The expression of p16INK4a (hereafter referred to as p16) is an optimal biomarker for both cellular senescence and biological ageing. p16 is located on chromosome 9p21 in close proximity to two other tumour suppressor genes, p15 and ARF, in humans. The p16 and ARF genes are alternatively spliced from the same locus, but the resulting protein sequences are different since their first exons are different [9].

p16 can bind to CDK4 and CDK6, which is essential in cell cycle control. To phosphorylate pRB, CDK4/6 commonly forms a protein complex with cyclin D. As pRB is phosphorylated, it separates from the transcription factor E2F1, allowing E2F1 to enter the nucleus and induce the transcription of target genes that help the

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cell transition from G1 to S process. As a result, p16 functions as a CDK inhibitor by blocking its association with cyclin D, thus preventing cell cycle progression [10,11]. Downregulation of p16 can result in cancer by disrupting cell cycle progression. The p16 gene is often mutated in many cancers, and p16 expression suppression is linked to an increased risk of cancer [12].

HR-HPV infection is associated with strong and diffuse cytoplasmic and nuclear expression of p16 in cervical squamous cell carcinomas. As a result, p16 is regarded as a proxy marker for chronic HR-HPV infection, and p16 over-expression has been found in the majority of cervical pre-cancers and cancers [13,14].

Characteristics features of Ki-67:

The Ki-67 protein has been commonly used as a proliferation marker for human tumour cells. It is also known as MKi-67. It is encoded by the MKI67 gene. It is solely related to cell proliferation. During interphase, the Ki-67 antigen is only found in the cell nucleus, while during mitosis, the majority of the protein is found on the chromosomes' surfaces. Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but it is absent in quiescent (resting) cells (G0).

Ki-67, a cell proliferation marker, predicts tumour malignancy. Ki-67 immunohistochemical analysis is usually conducted on paraffin-embedded sections and acts as an appealing index for prognosis and prediction in many tumours [15]. Ki-67 identification has been extensively used in the auxiliary diagnosis of cervical precancers and cancers [16,17].

Significance of p16 and Ki-67 dual-staining:

As we have already seen above, p16 is a tumor suppressor marker and Ki-67 is a cell proliferation marker. Under physiological conditions, p16 overexpression and Ki-67 expression are mutually exclusive and do not occur in the same cervical epithelial cell. As a result, p16/Ki-67 co-expression suggests HR-HPV-induced cell cycle deregulation, and identification of p16/Ki-67 co-expression will serve as a marker to predict HR-HPV-induced cell transformation and the existence of high-grade CIN lesions [18,19]. Antibodies against p16 and Ki-67 will detect p16/Ki-67 co-expression. p16 staining alone revealed the brown cytoplasm/nuclear signal, while Ki-67 staining alone revealed the red nuclear signal. Positive p16/Ki-67 dual-staining cells had brown cytoplasm signals for p16 expression, and dark red to red brown nuclear signals indicated p16 and Ki-67 co-localization in the same cell. Regardless of the morphological appearance of the cells, slides with one or more cervical epithelial cells stained for p16 and Ki-67 were classified as positive.

Positive p16/Ki-67 dual staining is linked to HR-HPV infection, specifically HPV 16 and 18 [20]. The positive rate for p16/Ki-67 in HPV-positive women was 78.9 percent, which was slightly higher than the 9.4 percent in HPV-negative patients [21] . When compared to cases infected with other HR-HPV forms, the correlation of p16/Ki-67 positivity with HPV16 and/or 18 infections was 2-4 times greater [22]. The presence of p16/Ki-67 dual staining also clearly suggests the presence of CIN2+ or high-grade squamous intraepithelial lesion (HSIL). Positive rates of p16/Ki-67 dual staining in HR-HPV positive women with diagnoses of NILM, ASCUS, LSIL, atypical

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squamous cells cannot exclude HSIL (ASC-H), and HSIL were 3.0 %, 23.6 %, 25.8 %, 78.6%, and 100.0 %, respectively [23]. The positive rating rose from 31% in women with negative

cytology to 92% in women with HSIL 41. Similarly, the positive rate of p16/Ki-67 in women with CIN3 was 86 percent, which was considerably higher than the rate in women without biopsy findings, which was 24 percent. P16/Ki-67 double staining was used in all cervical cancer patients. The sensitivity and specificity of p16/Ki-67 dual staining for CIN2+ is 74.9%-90.9% and 72.1%-95.20%, respectively. The positive rate of CIN2+ detected by p16/Ki-67 dual staining was 92.7 percent, which was higher than the 71.1 percent detected by HPV16/18 genotyping alone. On comparison with other methods available, p16 and Ki-67 dual staining has the higher sensitivity and specificity for detection and can considerably reduce the number of patients referred forcolposcopy, especially in young women with a high HPV infection risk.

Among so many various types of cancer, cervical cancer is the only one in the world, for which causes and preventive measures are known. There are many studies which show that cervical cancer occurs with infection of HPV, that's why HPV screening has its own significance.

For screening followed by early detection, p16 is the major participant of regulatory normal cell cycle. It gives a significant value for excess cervical lesions. P16 protein expresses itself in ⅓ and ⅔ layers of cervical squamous epithelium. It has been confirmed that p16 is correlated with degree of cervical lesions.

Another biomarker, Ki-67 indicates abnormal cell proliferation. It cannot be detected in normal cervical tissues but can be detected with increased amounts of cervical lesions.

Basically, it can be summarized as p16 and Ki-67 has a very high cross sectional sensitivity. Usage of p16 and Ki-67 efficiently and safely reduces colposcopy with higher reduction seen in 30 years and older. Reduced colposcopies leads to cost effective testing in this process. It can be a milestone in the path of cancer research.

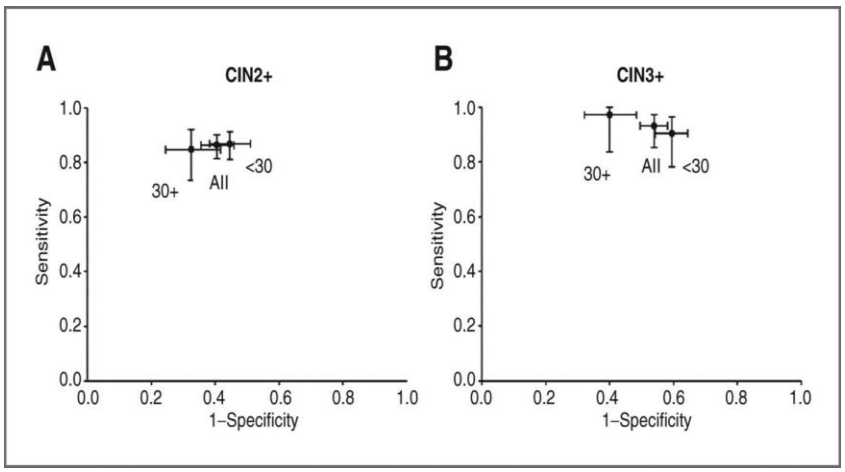

Fig 2: Graph showing the specificity v/s sensitivity among CIN2+ and CIN3+.

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CONCLUSION:

Till now, there are many biomarkers being investigated with great potential in diagnosis of cancer. Among them, p16 and Ki-67 biomarkers have a significant role in screening of cervical cancer. It facilitates us with a good risk marker for the stratification of HPV positive women which also includes normal cytological patients. This provides higher sensitivity and specificity with improved diagnosis of cervical cancer and its precancerous lesions.

It is noted that in some cases, results of p16 and Ki67 are hard to analyze. Till date, it

should be believed that p16 and Ki67 have broad range of applications in diagnosis and treatment of cervical cancer and triaging of cervical cancer and precancer lesions as well.

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All the authors gathered the data, analysed it and designed amd contributed to the final manuscript.

Conflict of Interest:

Conflict of interest declared none.

References

1. Medicalnewstoday.com. 2021. *Cancer: Overview, causes, treatments, and types*. [online] Available at: <https://www.medicalnewstoday.com/articles/323648>
2. National Cancer Institute. 2021. *Understanding Cancer*. [online] Available at: <https://www.cancer.gov/about-cancer/understanding> [Accessed 22 May 2021].
3. Cdc.gov. 2021. *Basic Information About Cervical Cancer | CDC*. [online] Available at: <https://www.cdc.gov/cancer/cervical/basic_info/index.htm> [Accessed 22 May 2021].
4. Training.seer.cancer.gov. 2021. *Introduction to Cervical Cancer | SEER Training*. [online] Available at: <https://www.training.seer.cancer.gov/cervical-uterine/cervix/intro/> [Accessed 22 May 2021].
5. National Cancer Institute. 2021. *HPV and Cancer*. [online] Available at: <https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-and-cancer>
6. TruScreen. 2021. *Cervical Cancer Screening Methods | TruScreen*. [online] Available at: <https://truscreen.com/medical-science/current-screening-methods/>
7. GoMedii Blog. 2021. *Screening Methods Of Cervical Cancer: Every Woman Must Know - GoMedii*. [online] Available at: <https://gomedii.com/blogs/english/health-a2z/screening-methods-of-cervical-cancer/>
8. TruScreen. 2021. *Cervical Cancer Screening Methods | TruScreen*. [online] Available at: <https://truscreen.com/medical-science/current-screening-methods/>
9. Muss, H. B. et al. (2020) "P16 a biomarker of aging and tolerance for cancer therapy," Translational cancer research, 9(9), pp. 5732–5742.
10. Kalof, A.N. and Cooper, K., 2006. p16INK4a immunoexpression: surrogate marker of high-risk HPV and high-grade cervical intraepithelial neoplasia. *Advances in anatomic pathology*, *13*(4), pp.190-194.

11. Cicenas, J., Kvederaviciute, K., Meskinyte, I., et al. 2017. KRAS, TP53, CDKN2A, SMAD4, BRCA1, and BRCA2 mutations in pancreatic cancer. *Cancers*, *9*(5), p.42.

12. Leversha, M.A., Fielding, P., Watson, S., et al. 2003. Expression of p53, pRB, and p16 in lung tumours: a validation study on tissue microarrays. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *200*(5), pp.610-619.

13. Yu, L., Fei, L., Liu, X., et al. 2019. Application of p16/Ki-67 dual-staining cytology in cervical cancers. *Journal of Cancer*, *10*(12), p.2654.

14. Yu, L., Wang, L., Zhong, J. et al. 2010. Diagnostic value of p16INK4A, Ki-67, and human papillomavirus L1 capsid protein immunochemical staining on cell blocks from residual liquid-based gynecologic cytology specimens. *Cancer cytopathology*, *118*(1), pp.47-55.

15. Menon, S.S., Guruvayoorappan, C., Sakthivel, K.M., et al. 2019. Ki-67 protein as a tumour proliferation marker. *Clinica Chimica Acta*, *491*, pp.39-45.

16. Silva, D.C., Goncalves, A.K., Cobucci, R.N., et al. 2017. Immunohistochemical expression of p16, Ki-67 and p53 in cervical lesions–A systematic review. *Pathology-Research and Practice*, *213*(7), pp.723-729.

17. Petry, K.U., Schmidt, D., Scherbring, S., et al. 2011. Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology. *Gynecologic oncology*, *121*(3), pp.505-509.

18. Ikenberg, H., Bergeron, C., Schmidt, D., et al. 2013. Screening for cervical cancer precursors with p16/Ki-67 dual-stained cytology: results of the PALMS study. *Journal of the National Cancer Institute*, *105*(20), pp.1550-1557.

19. Yu, L.L., Chen, W., Lei, X.Q., et al. 2016. Evaluation of p16/Ki-67 dual staining in detection of cervical precancer and cancers: a multicenter study in China. *Oncotarget*, *7*(16), p.21181.

20. Uijterwaal, M.H., Witte, B.I., Van Kemenade, F.J et al. 2014. Triaging borderline/mild dyskaryotic Pap cytology with p16/Ki-67 dual-stained cytology testing: cross-sectional and longitudinal outcome study. *British journal of cancer*, *110*(6), pp.1579-1586.

21. Donà, M.G., Vocaturo, A., Giuliani, M, et al. 2012. p16/Ki-67 dual staining in cervico-vaginal cytology: correlation with histology, Human Papillomavirus detection and genotyping in women undergoing colposcopy. *Gynecologic oncology*, *126*(2), pp.198-202.

22. Rossi, P., Borghi, L., Ferro, R. et al., 2015. A population of 1136 HPV DNA-HR positive women: expression of p16 (INK4a)/Ki67 Dual-Stain Cytology and cytological diagnosis. Histological correlations and cytological follow up. *pathologica*, *107*(3-4), pp.185-191.

23. Ordi, J., Sagasta, A., Munmany, M et al., 2014. Usefulness of p16/Ki67 immunostaining in the triage of women referred to colposcopy. *Cancer cytopathology*, *122*(3), pp.227-235.