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Advancement in Diagnosis of Ovarian Cancer

Jyoti Sijwali[1], Dr. Anjana Goyal[2], Jai[3]
[1]*Research Scholar Department of Biochemistry, MRIIRS Faridabad, Haryana*
[2]*Professor & Head, Department of Biochemistry, MRIIRS Faridabad, Haryana*
[3]*Research Scholar Department of Biochemistry, MRIIRS Faridabad Haryana*

Abstract: ***Epithelial ovarian cancer is usually found in the advanced stage. The current state of the art surgery and chemotherapy leads to complete release; However, the repetition rate is high. in many patients, the disease ends up being a continuous period without symptoms and episodes of recurrence. A variety of targeted therapies and biological drugs, currently under development, offer the promise of transforming ovarian cancer, the latest major studies in a variety of new conventional therapies, and new therapies, recently approved and / or clinical trials.***

Human papillomavirus (HPV) circulating tumour DNA (HPV ctDNA) was proposed as a biomarker for the detection and monitoring of HPV-related cancer infections. one hundred and eight plasma samples were obtained from women diagnosed with HPV-16 positive (CC) (n = 100) cervical cancer, pre-HPV16 (cervical intraepithelial neoplasia grade3 (CIN3) (n = 20), and controls non-HPV DNA (n = 60) randomly selected from the archives to evaluate the effectiveness of the bead-based HPV genotyping assay (E7 type-specific multiplex genotyping assay (E7-MPG) in detecting HPV16 ctDNA. E7-MPG performance compared and those for DNA detection by droplet digital PCR (ddPCR) and HPV16 E16 antibody detection in independent studies Internal control for DNA quality testing is included in molecular testing, i.e., beta-globin and ESR1, respectively.

The sensitivity and specificity of E7-MPG and / or E6 antibodies for CCV16-positive CC were tested. HPV16 ctDNA was detected using E7-MPG in 42.3% of all plasma samples and 7.4% of plasma samples from HPV16-positive CC conditions. E7-MPG test confirmation of HPV16-positive Cervical Cancer detection was higher than that of ddPCR (74.7% vs 63.1%, P <0.001). when considering both HPV16 ctDNA and E6 antibodies, sensitivity to HPV16-positive CC detection increased from 74.7% to 86.1%, while specificity did not change to 97.8%. E7-MPG activation for HPV16 ctDNA appears to be at least as sensitive as that of ddPCR, providing an additional ctDNA detection tool for HPV infection, similar to the E6 antibody, further improving Cervical Cancer detection.

Cervical cancer is caused by progressive infections with the oncogenic human papilloma virus (HPV) While HPV infection. It is an important factor; it is not enough to cause cancer. Most HPV infections cause pre-existing low-grade ulcers that are automatically erased after a few months in more than 90% of cases, and less than 10% eventually progress to advanced levels or advanced cancer. Progress is evident in the uncontrolled exposure of the virus oncogene E6 and E7 to basal and parabasal infested cells. Novel biomarkers that allow monitoring of these important cellular events in histological or cytological specimens may improve the detection of lesions with a high risk of progression in both basic tests and measurement settings. In this review, we will discuss the possible biomarker symptoms of cervical cancer screening by focusing on the level of clinical evidence supporting their application as marking novels in refined cervical cancer screening programs.

Keywords: Cervical cancer, Screening, Biomarker, HPV, E6, E7

I. INTRODUCTION

The introduction of the Papanicolaou (PAP) cervical examination by George Papanicolaou in the 1920s has dropped dramatically rates of morbidity and mortality of cervical cancer. However, limitations regarding its sensitivity and specificity have prompted a search for specific biomarker markers for dysplastic squamous and glandular cells of the cervix. I hope these biomarkers can be used collaboratively. This standard testing programs to improve diagnostic and reproductive consistency.

A. Author Note

Jyoti Sijwali[1,] Dr. Anjana Goyal[2,] and Jai[3] Research Scholar Department of Biochemistry, MRIIRS Faridabad, Haryana[1], Professor & Head, Department of Biochemistry, MRIIRS Faridabad, Haryana[2], Research Scholar Department of Biochemistry, MRIIRS Faridabad Haryana[3],
We have no known conflict of interest to disclose correspondence concerning this article should be addressed to Jyoti Sijwali Research Scholar, Department of Biochemistry, MRIIRS, Faridabad
E-mail: jyotisijwali@gmail.com

Biomarkers will allow to compare the effectiveness of human papillomavirus (HPV) vaccines and will help expose new mechanisms involved in the pathogenesis of cervical dyskaryosis. HPV contributes to neoplastic progression mainly through the action of two viral oncoproteins — E6 and E76 and is characterized by changes in host cell production cycle control proteins. Such differentiated proteins as nucleic acids may play a role as 'biomarkers' of dysplastic cells. To date, a variety of cell markers have been tested. Three major markers of cyclin dependent kinase inhibitor p16INK47–9 and DNA replication replication proteins CDC6 (cell division cycle protein 6) and MCM5 (mini chromosome maintenance 5).

The genetic product of CDKN2A, p16INK4A, is a tumour suppressor protein that inhibits cyclin dependent kinases 4 and 6, forming a phosphorylate retinoblastoma (Rb) protein. A coherent correlation between p16INK4A and Rb expression was found, suggesting the existence of a negative feedback loop that allows the Rb to limit p16INK4A levels. p16INK4A Excessive stress has been shown in cervical cancer, and is thought to be the result of Rb dysfunction of the HPV E7 protein.17 p16INK4A may be direct acquired by transcription factor E2F, which is released from the Rb protein after the binding of HPV E7. The p16INK4A expression pattern in dysplastic squamous as well cervical cells in parts of the tissue and in the cervix smears thoroughly investigated. It is now widely accepted that p16INK4A is a sensitive and direct marker of squamous cells and dysplastic cervical cancer, and is an important component of a combination of cervical cancer ulcer diagnosis and cervical examination. In all eukaryotes, there is a preserved mechanism of DNA replication, which ensures that DNA replication occurs only once in a single cell cycle. DNA replication requires a controlled integration of pre replicative complexes.

DNA replication requires a controlled combination of replica compounds in DNA during the G1 phase of the cell cycle. Repetitive structures make chromatin stronger or ''licensed'' to replicate. Among the proteins known to combine to form pre replicative complexes are CDC6 and mini chromosome maintenance (MCM) proteins. Decreased regulation of DNA replication results in genomic instability and contributes to malignant cell mutations. Not surprisingly, increased expression of MCM5 and CDC6 appears on dysplastic cells. In normal cells, MCM5 and CDC6 exist only during the cell cycle and are lost in the cell during silence and separation. As a result, MCM5 and CDC6 are specific biological markers of growing cells. Several studies have highlighted their potential use in the diagnosis and / or diagnosis of various dysplastic and neoplastic conditions. The purpose of our study was to compare and contrast the expression patterns of p16INK4A, MCM5, and CDC6 and to evaluate their potential as markers of dysplastic squamous cells and cervical glands. Immunohistochemical analysis of MCM5, CDC6, and p16INK4A was performed using mouse antibodies on formalized samples and paraffin wax embedded in the common cervix, cervical intraepithelial neoplasia 1 (CIN1), CIN2, CIN2, CIN2, CIN2 cervical glandular intraepithelial neoplasia cervical squamous carcinoma invasive, and adenocarcinoma. The expression p16INK4A, MCM5, and CDC6 was also analysed in a series of Thin Prepare smears showing mild, moderate, and severe dyskaryosis. HPV detection is performed through a modified SYBR green test system, using the first set of ''degenerate'' which detects sequences within the L1 study framework for at least nine HPV strains. Fluorogenic polymerase chain reaction (PCR) and PCR solution phase were used for some type of HPV type.

II. MATERIALS AND METHODS

Concentrated Formalin and embedded paraffin wax of cervical biopsy samples selected from Coombe pathology files Women's Hospital, Dublin, Ireland. Haematoxylin and eosin-coloured slides of all biopsy samples reviewed by a pathologist and classified according to criteria defined by the World Health Organization. Approval of the ethics of the use of all models was obtained from the research ethics committee of Coombe Women's Hospital. In total, 20 normal uterus selected biopsies, over 38 CIN1, 33 CIN2, 46CIN3, 10 invasive squamous cell carcinomas, 19 cGINs, and 10 adenocarcinomas. Because some biopsies could not be tested for all antigens, the numbers in some comparisons are small there are a number of biopsies available. Cells from C33A (HPVnegative), HeLa (HPV-18 positive), and CaSki (HPV-16) positive) lines of cervical carcinoma cells were collected in the middle Preserve Cyst liquid preserves and is used to repair Thin Prepare slides (Cytyc Corporation, Boxborough, Massachusetts, USA).

A. HPV Detection and Typing

After wax is extracted, the DNA is extracted using a Gentra DNA separation kit (Puregene, Minneapolis, Minnesota, USA), in accordance with the manufacturer's guidelines. The total amount of extracted DNA was performed using a real-time Taqman PCR test for real-time b actin (Applied Biosystems, Foster City, California, USA). Extensive HPV detection of HPV was performed using a modified SYBR green test system (Applied Biosystems). The standard HPV ''degenerate'' primer set (GAP 1 & 2), states find sequence within L1 open reading framework at least HPV types 6, 11, 16, 18, 30, 31, 32, 33, and 39 used. Fluorogenic PCR was then used for HPV type 16, 18, 31 and 33 detection and typing. The PCR solution phase is used by typing HPV-6 and HPV-11. Response situations once the primer pair and the probe sequence are described earlier.

B. Antibodies

Mouse antibodies are commercially available p16INK4A (PharMingen, San Diego, California, USA), MCM5 (Novacastra, Newcastle upon Tyne, UK), and CDC6 (Santa Cruz Biotechnology, Inc., Santa Cruz, California, USA) used.

C. Separation of p16INK4A / MCM / CDC6 antibodies by western blot analysis

CaSki cells are homogenised to RIPA buffer (Santa Cruz Biotechnology). Protein extracted and separated by electrophoresis in 15% sodium dodecyl sulphate polyacrylamide electrophoresis gel and transferred to the nitrocellulose membrane using the transmission bath containing 25mM Tris / HCl, pH 8.3, 192mM glycine, and 20% methanol. Blot previously blocked by 3% Marvel in TTBS (20mM Tris / HCl, pH 7.5, 0.5M NaCl, 0.05% vol / vol Tween 20). The blot was then combined with a pure antihuman mouse p16INK4A, MCM5, or CDC6 monoclonal antibody. After three 10-minute baths on PBST (136mM NaCl, 26mM KCl, 15mM KH2PO4, 82mM Na2HPO4, 0.05% vol / vol Tween 20) the blot was then placed in biotinylated universal secondary antibody, Vectatain Antibody ABC, Vectain Laboratories California, USA) 30 minutes. This was followed by three baths in PBST and incubation in the avidin – biotin complex (Vectastain ABC kit; Vector Laboratories) for 30 minutes. Immunoreactive bands were obtained with diaminobenzidine (Vector Laboratories).

D. Immunocytochemistry on Biopsy Samples

The sections (size 4 mm) were cut into prepared formalin paraffin wax embedded biopsy samples and mounted on 3-aminoproplytriethoxysilane composite glass slides. Categories waxed using xylene and then re-extracted from high-quality alcohol. Endogenous peroxidase activity blocked by incubating phases at 0.3% H2O2 / methanol for 30 minutes. Antigen regeneration was performed at 0.01M citrate buffer (pH 6) using a pressure cooker way. After washing the sections in Tris protected from excess salt (pH 7.4) indirect immune binding was reduced by incubation stages in 0.1% of bovine serum albumin 30 minutes. After excessive serum extraction, phases were performed incubated one hour at room temperature and cleaned anti-human mouse p16INK4A (1/75 dilution; PharMingen), MCM5 (1/40 dilution; Novacastra), or CDC6 (1/40 dilution; Santa Cruz Biotechnology) antibody monoclonal. After a good bath with Tris protected saline, stages installed biotinylated universal secondary 30 antibodies (Vectastain ABC kit; Vector Laboratories) minutes. This was followed by avidin incubation- biotin complex (Vectastain; Vector Laboratories) 30th minutes. Slides developed with diaminobenzidine counter stain hematoxylin slightly.

E. Immunocytochemistry on Thin Prepare slides

The process of immunocytochemical analysis in Thin Preps was similar to the process described above for immunohistochemical analysis, except that the dissolution of waxing. step in xylene and antigen recovery steps were not performed. Thin Prepare of CaSki, HeLa, and C33A cells were used as good controls to test each running specification.

F. Interpretation of p16INK4A/CDC6/MCM5 Expression in Biopsies Tissues

All embedded parts of formalin and paraffin embedded showed either Strong nuclear or cytoplasmic staining it is considered good. The certified pathologist then prescribed all the graded categories according to quality according to the following random scale: 0 (no good stain of dysplastic cells), 0a (basal layer staining), 1 (basal layer staining plus, 10% good staining of dysplastic cells), 2 (. 10% but, 50% good colour of dysplastic cells), and 3 (50% good positive staining of dysplastic cells.

III. RESULT

A. HPV Detection and Typing

SYBR green HPV analysis detects CaSki and HeLa cell lines good on HPV DNA. Additional HPV typing by Fluorogenic PCR found that CaSki cell line contains HPV-16 DNA sequence, whereas the HeLa cell line contains HPV-18 DNA sequence. C33A for cervical cancer line it was found to be free of HPV DNA with raw SYBR HPV analysis. All cases are approved for HPV using SYBR green PCR they also had HPV in some form of PCR. describe the relationship between HPV status and p16INK4A, MCM5, and CDC6 resistance.

B. Manifestation of Monoclonal Antibodies

Appearance of the mouse monoclonal anti-p16INK4A, anti-MCM5, and anti-CDC6 antibodies was made by westerner's blot analysis of total protein extracted from CaSki cells. Analysis of the Western blot revealed that all three antibodies were present clear with no indirect bands present. 16 kDa, 82 kDa, and 62.7 kDa bands, corresponding to the p16INK4A, MCM5, and CDC6 proteins, respectively.

C. Immunocytochemical Staining of p16INK4A / MCM5 / CDC6 in Histologically Normal tissue Samples

Immunocytochemical analysis using a monoclonal anti-p16INK4A antibody was performed at 20 histologically normal cervical biopsies. In all normal epithelial, stromal, metaplastic, reactive, and inflammatory cells were spotless. Negative immunostaining with the anti-p16INK4A antibody was also observed in the glandular endocervical epithelium. The antibody was seen in the glandular endocervical epithelium. In addition, the common areas around CIN lesions did not express p16INK4A. Immunostaining uses the monoclonal anti MCM5 antibody to produce strong nuclear stains on the growing parts of the cervical squamous epithelium. In all 20 standard biopsies tested. This type of contamination of the extensor basal layer is given points as 0a. Contamination of the separating layers of the top layer was not present in all the tested conditions. A small number of common biopsies showed occasional contamination of inflammatory cells in the cervical stroma and contamination between endocervical glands. Immunostaining using monoclonal anti-CDC6 antibody was negative in 18 of 20 common cases, and two showed a weak nuclear contamination of the growing basal layer (staining intensity of 0a). Common areas around CIN and cGIN lesions, in addition to metaplastic, stromal, reactive, and inflammatory cells were negative for CDC6 expression.

D. Immunocytochemical Staining for p16INK4A in the cervix ulcers

Dysplastic epithelial cells showed a strong p16INK4A concentration of 38 of 38 CIN1, 33 of 33 CIN2, 45 of 46 CIN3, and 10 of 10 invasive squamous cell carcinoma samples. A clear difference was observed between dysplastic cells and adjacent normal cells. Interestingly, a small number of CIN1 cases showed specific nuclear contamination, although in the remaining CIN1, CIN2, and CIN3 lesions and squamous cell carcinomas a combination of nuclear and cytoplasmic staining was observed. A simple retrospective line analysis reveals the most important line correlation (moderate slope, 0.46; R2 = 0.32; p, 0.0001) between p16INK4A contamination and the increasing range of squamous dysplasia. In all 19 cases of cGIN tested, it was possible that cytoplasmic staining or a combination of cytoplasmic staining and nuclear detection were detected. There are no cGIN cases showing only the nuclear signal. All 10 adenocarcinomas showed nuclear intensity and cytoplasmic staining. In our study, p16INK4A had a median 3rd grade in all squamous lesions and dysplastic glands and in all squamous cell carcinomas and adenocarcinoma tested. A strong correlation between HPV positivity and p16INK4A staining intensity is evident. Positive strains of p16INK4A that had a higher risk of HPV 16 or 18 strains have a higher risk of staining than negative HPV strains or conditions with low HPV strains 6/11.

E. Immunocytochemical Staining for MCM5 in Cervical Lesions

The dynamic nuclear spectrum of MCM5 was detected in 29 of 31 CIN1, 24 of the 25 CIN2s, and 35 of the 38 CIN3 lesions tested. One isolated case showed medial endocervical glandular cells. Occasional contamination of stromal cells was it appears in a few isolated cases. MCM5 immunostaining for CIN lesions. All 10 squamous cell carcinomas exhibited the intense nuclear staining of MCM5. A simple linear regression analysis revealed a significant linear regression between MCM5 staining and the degree of squamous dysplasia (moderate slope, 0.59; R2 = 0.26; p, 0.0001). Strong immunity to MCM5 protein was also observed in 14 of the 17 cases of cGIN and all 10 adenocarcinomas. Glandular ulcers and adenocarcinomas showed a prominent pattern of nuclear stains. Cytoplasmic staining was not detected. MCM5 had a median degree of contamination 2 for all stages of squamous dysplastic ulcers and glandular dysplastic ulcers. In invasive squamous cell carcinoma and adenocarcinoma, the median concentration level was 3. There was no obvious correlation between high risk of HPV positivity and MCM5 range of computational power. Between the CIN1, CIN2, and CIN3 diagnostic groups, the level of stains appeared to be independent of the high-risk HPV.

F. Immunocytochemical Staining for CDC6 in cervical Lesions

CDC6 anointing was seen in 20 of 32 CIN1, 17 of 27 CIN2, 25 of the 33 CIN3s, and eight of the eight squamous cell carcinoma cases. A pattern of nuclear staining was observed in all dysplastic ulcers, and other high-grade ulcers cytoplasmic blush. All eight squamous cell carcinomas tested had CDC6 protein expression. The pattern of nuclear staining has been shown to be squamous cell carcinomas, staining. A simple retrospective line analysis reveals that some conditions indicate a very important cytoplasmic line (average volume, 0.7; R2 = 0.2; p, 0.0001) between CDC6 staining and increased intensity of cervical dysplasia. This is the middle distance 1 was found in all marks of squamous dysplastic lesions, and the median incidence of invasive squamous carcinomas was 3. Eleven of the 14 cGINs showed degeneration of dysplastic nuclei, with a median grade 2. Severe nuclear and cytoplasmic staining has been detected in seven of 10 cases of adenocarcinoma, with a median 2nd grade.

As previously described by Bonds et al, half of the cells undergo exposure to CDC6 proteins. increased with increasing degree of dysplasia.10 Among the areas of dysplasia, a strong correlation between CDC6 positivity and histological evidence of HPV was noted. Overall, a strong correlation between the high risk of HPV positivity and the presence of CDC6 was observed. However, with respect to MCM5, there was no clear correlation between high HPV positivity risk and CDC6 specificity.

G. Immunocytochemical staining for p16INK4A/MCM/CDC6 in cell lines

Examining the specificity of pure monoclonal mouse antihuman p16INK4A, MCM5, and CDC6 antibodies, immunostaining was performed on CaSki cells asynchronous (HPV-16 positive), HeLa (HPV-18 positive), and C33A (HPV negative). CaSki positive HPV-16 cells and HeLa positive HPV-18 cells showed strong immune defenses against all three antigens. The HPV negative C33A (ATCC) cell line was also excellent for the expression of p16INK4A, MCM5, and CDC6 proteins. MCM5 showed a strong pattern of nuclear stains across all cell lines, while p16INK4A and CDC6 showed a combination of nuclear and cytoplasmic staining. This type of CDC6 nuclear and cytoplasmic expression in the cervix lines of carcinoma cells are consistent with previous studies.

H. Immunocytochemical Staining for p16INK4A in Thin Prepare smears

Immunocytochemical analysis using monoclonal anti-p16INK4A was performed on a series of Thin Prepare slides. The antibody test for p16INK4A was positive for all five smears showing mild dyskaryosis, six out of seven smears showing moderate dyskaryosis and all eight slides showing severe dyskaryosis. The p16INK4A antibody assay was not included in all 12 regular smears tested.

I. Immunocytochemical staining for MCM5 in Thin Prepare smears

The MCM5 antibody test was present in one of the five smears showing mild dyskaryosis, three out of five smears show moderate dyskaryosis, as well as four out of five smears indicate worse dyskaryosis. MCM5 staining was very clear nuclear. The MCM5 antibody test was also positively immature metaplastic cells in one of the 10 standard smears tested.

Immunocytochemical Staining for CDC6 in Thin Prepare smears

Colour CDC6 patterns and local cellular performance were present tested in Thin Prepare slides series. CDC6 antibody the assay showed a blurred nuclear power in one of the five smears with central dyskaryosis and one of five smears precentral dyskaryosis. Two out of five smears show firmness. Severe nuclear positivity dyskaryosis was identified. One in 10 regular smears tested show blurred spots immature metaplastic cells.

IV. DISCUSSION AND RESULT

A wide range of potential biomarkers has been tested diagnostic assistance in diagnosing cervical cancer and its precursors. The three most potent markers are the cyclin dependent kinase inhibitor p16INK4A4 and DNA replication replication proteins MCM5 and CDC6.The purpose of our study was to compare and contrast the expression patterns of these three proteins in normal epithelium, dysplastic squamous and glandular lesions, squamous carcinoma, and adenocarcinoma and oral examination. their diagnostic assistance as biomarkers for cervical dysplasia. We found that all three markers indicated a linear relationship between their presence or absence and the degree of dysplasia. However, differences were found between the diagnostic usefulness of the three markers. Of the three tested models, p16INK4A showed a very large diagnostic tool. Our findings clearly support many other reports confirming the hypothesis that p16INK4A is overexpressed to squamous cells and dysplastic glands of the cervix and is a useful compound test that is useful in wound diagnosis and cervical screening. We found a strong correlation between HPV positivity and p16INK4A positivity, although it should be noted that p16INK4A manifestations were also observed in a limited number of HPV strains bad cases. These lesions often indicate p16INK4A deficiency. Milde-Langosch et al also reported P16Ink4A is excessive stress at 41% of HPV negative adenocarcinoma. This discovery, complying with the HPV negative C2A, which can indicate that the rating method is not HPV e7 of P16Ink4A Rival may be available. Loss of compression writing prior to inactive changes in the Rb gene is a well-defined HPV-related regulation of p16INK4A. Indeed, the p16INK4A expression in some cases may be independent of pRb. Henshall et al suggested that further growth, despite the effects of inhibition of p16INK4A expression, may occur if cells are also overexpressed cyclin E or if p27Kip is not available.

The key was to detect one HPV CIN3 negative lesion was negative for p16INK4A protein a quote but good for both MCM5 and CDC6. One possible explanation for the absence of the term p16INK4A in this wound may be methylation of the p16INK4A promoter, leading to genetic mutation of p16INK4A.

The promotional hypermethylation of p16INK4A may be an alternative leading to disruption of Rb – p16INK4A administration in independent HPV cervical dyskaryosis. This case shows that, although rare, the cervical p16INK4A is negative Dyskaryosis occurs. This may have a significant impact on the use of p16INKA colour as a 'stand-alone test', and supports the use of compounds for cervical symptoms dyskaryosis. p16INK4A is strongly expressed in dysplastic cells of the cervical gland. However, in addition to seeing cGIN, in order to be an effective biomarker, it is also important that p16INK4A should differentiate these lesions from mammary gland lesions, such as tubo-endometrioid metaplasia. may limit the use of p16INK4A as a stand-alone biomarker for the diagnosis of cervical glandular dysplasia.46 As suggested by Cameron et al, p16INK4A should be used in combination with other biomarkers.

''Of the three markers assessed, p16INK4A showed the greatest diagnostic utility''

The MCM5 showed a strong correlation between its expression and the stage of dysplasia. We also saw it from time-to-time contamination between stromal cells and normal endocervical glands. The light separating cells were negative in all tested conditions. Strong immune system was observed in all squamous cancer invasive and in 88 of the 94 CIN cases tested. Of the thyroid glands tested, 14 of the 17 cGIN cases were positive in MCM5 expression. All adenocarcinoma cases tested were positive for MCM5 protein expression. MCM5 also contaminates exfoliated dysplastic cells within Thin Prepare slides and may be as powerful as a tag exfoliated cells show moderate and severe dyskaryosis. Abnormal contamination of normal metaplastic cells was found. However, these cells can be easily identified by a morphological process using the colour Papanicolaou. A large-scale study of MCM5 staining on Thin Prepare smears is currently underway. This may be due to the release of Rb inhibition in E2F by binding to HPV E7 oncoproteins. The transcription factor E2F facilitates the promotion of MCM5 transcripts by binding to MCM5 developer sites. A high rate of HPV infection at high risk was present at all stages of dysplasia in the study. Because Coombe Women's Hospital is in a high-risk area and is a center for advanced cervical transmission this is not expected. Interestingly, however, there was no significant correlation between MCM5 staining intensity and HPV status between diagnostic phases. As suggested by Davidson et al, this lack of correlation between HPV positivity and robustness indicates that the expression of MCM5 proteins is a rate of increase and independence without their presence of HPV. Indeed, MCM5 enhancement is described in a variety of HPV-related neoplasms, indicating that although in the cervical MCM5 it may be the result of HPV infection, it does not depend solely on it. This highlights the potential of MCM as a biomarker in both HPV-dependent and independent HPV cervical dysplasia. This may be especially important in the case of cervical ulcers, a pathogenesis that appears to be less commonly associated with HPV infection.

CDC6 has shown a strong correlation between its claims and existing degree of dysplasia. In C33A, HeLa, and CaSki lines of cervical carcinoma cells, CDC6 showed strong nuclear and cytoplasmic staining, consistent with the previous one lessons. Using immunofluorescent staining, **Fujita et al.,** demonstrated the nuclear and cytoplasmic contamination of CDC6 asynchronous HeLa cervical carcinoma cell. Fujita et al reported that approximately 60-70% of cells exhibit strong nuclear contamination, with cytoplasmic positivity, while remaining interphase cells exhibit strong cytoplasmic resilience. C33A cells. In our study, a prominent pattern of nuclear stains was observed in all dysplastic lesions, with high-grade lesions and squamous cell carcinoma showing cytoplasmic decay. After phosphorylation with cyclin A-cyclin dependent kinase 2, the CDC6 protein is transported from its chromatin sites to the cytoplasm during the replication phase (S-phase) of the cell cycle. CDC6 and is degraded by ubiquitin dependent proteolysis by anaphase-promoting complex / cyclosome. Removal of CDC6 from the cytoplasm prevents recurrence and is required for the combination of phase S and subsequent mitosis.53 54 The presence of CDC6 cytoplasmic staining in several high-grade lesions and squamous cell carcinomas is likely to result in the accumulation of cytoplasmic staining. CDC6 proteins in the cytoplasm after repeated and long S levels in dysplastic cells.

Although CDC6 showed a significant correlation between the degree of contamination and dysplastic, CIN1 numbers and a small degree of positive CIN2 / CIN3 expression of CDC6 expression were significantly lower than previously described by William et al, who used another CDC6 polyclonal immune system. However, our results are well related to more a recent study published by Bonds et al, which used another mouse monoclonal anti-CDC6 antibody (Neomarkers, Freemont, California, USA) .10 Similar to us, they found a decrease in CDC6 positivity in low-grade lesions. Additionally, although the proportion of positive cells in CDC6 expression increased with increasing glandular range and squamous dysplasia, the proportion of positive dysplastic cells was lower compared to p16INK4A and MCM5. However, all squamous cell carcinomas tested showed strong nuclear capacity and specific cytoplasmic spots. CDC6 expression was present in 11 of the 14 cGINs and seven in 10 cases of adenocarcinoma. This is closely related to the Bonds et al study, in which 11 of the 14 adenocarcinomas in situ conditions and eight out of 10 adenocarcinomas were positive in CDC6 expression. as controller of the G2 / M test point. Degradation of CDC6 is required to enter the M phase of the cell cycle.55

This may explain why CDC6 is not detected or exposed at very low levels in early neoplastic cells. It is possible that as a result of neoplastic progression, neoplastic cells at higher levels may find a way to avoid the control of the CDC6 G2 / M phase, and thus continue to increase in the presence of increasing CDC6 protein levels. CDC6 immunostaining in Thin Prepare smears has shown that CDC6 expression is selectively selected from highly exfoliated dysplastic cells. These results are consistent with findings at the histological level. We found an association between CDC6 positivity and high HPV risk. However, in the case of MCM5, no correlation was found between high risk of HPV positivity and the degree of staining. Similar to Bonds et al, we found that CDC6 was specifically expressed in historical sites.

This may suggest activation of genomic DNA HPV recurrence processes associated with oncoproteins. Rb activation by HPV E7 removes E2F inhibition and may result in regulation of CDC6 transcription. A recent publication by Vaziri et al reported that excessive stress of CDC6 combined with Cdt1 promotes the replication of human cancer cells with p53 inactive but not to cells with p53.56 Active replication causes genomic instability and DNA damage, resulting in damage of DNA. testing methods to activate tumour suppressor protein p53. P53 prevents recurrence by inducing G1 cell cycle binding or otherwise by apoptosis. High-risk HPV E6 is targeted at p53 for proteolytic degradation. Therefore, HPV-infected cells have developed a strategy that allows for long-term replication, despite the presence of DNA damage and excessive exposure to CDC6 protein. Interestingly, the CDC6 expression pattern symbolizes high-risk glasses HPV E6 oncoprotein, which is strongly expressed in high-grade lesions and invasive carcinomas. These results suggest that although CDC6 undoubtedly plays an important role in malignant cell turnover, its potential role as a biomarker of cervical gland lesions and squamous dysplastic is limited in relation to low-grade lesions. CDC6 may act as a high-grade biomarker with invasive cervical sores.

‘‘The low or absent expression of CDC6 in low grade lesions may be a function of its role as a G2/M phase checkpoint regulator’’

In summary, all three markers examined in our study showed an important relationship between antibody contamination and the degree of dysplasia. Of the three potential biomarkers tested, p16INK4A proved to be the most reliable marker of cervical dysplasia. We found that p16INK4A marks all stages of squamous ulcers and cervical glands, and its expression was closely related to HPV at high-risk infection. However, the failure of p16INK4A to mark. The CIN3 case is isolated, as well as its reported staining of glandular mimics such as TEM, it can reduce its use as an independent test for cervical dysplasia. These results suggest the use of a combination of dysplastic symptoms in a difficult diagnosis charge. We found that MCM5 marked all squamous distances and cervical dysplastic cells of the cervix, as well as spots the severity appears to be independent of HPV at high-risk infection, which highlights the power of MCM as a biomarker both HPV-dependent and independent HPV of the cervix dysplasia. Excessive exposure to CDC6 protein has been observed in all marks for cervical dysplasia, but it was much better exposed to high levels and invasive lesions. Our results indicate that CDC6 may function as a top biomarker level and invasive cervical sores. Although our research has shown that p16INK4A, MCM5, and CDC6 can identify dysplastic cells exfoliated in cytology samples further research will be needed to accurately identify the role of each mark in a basic cervical examination.

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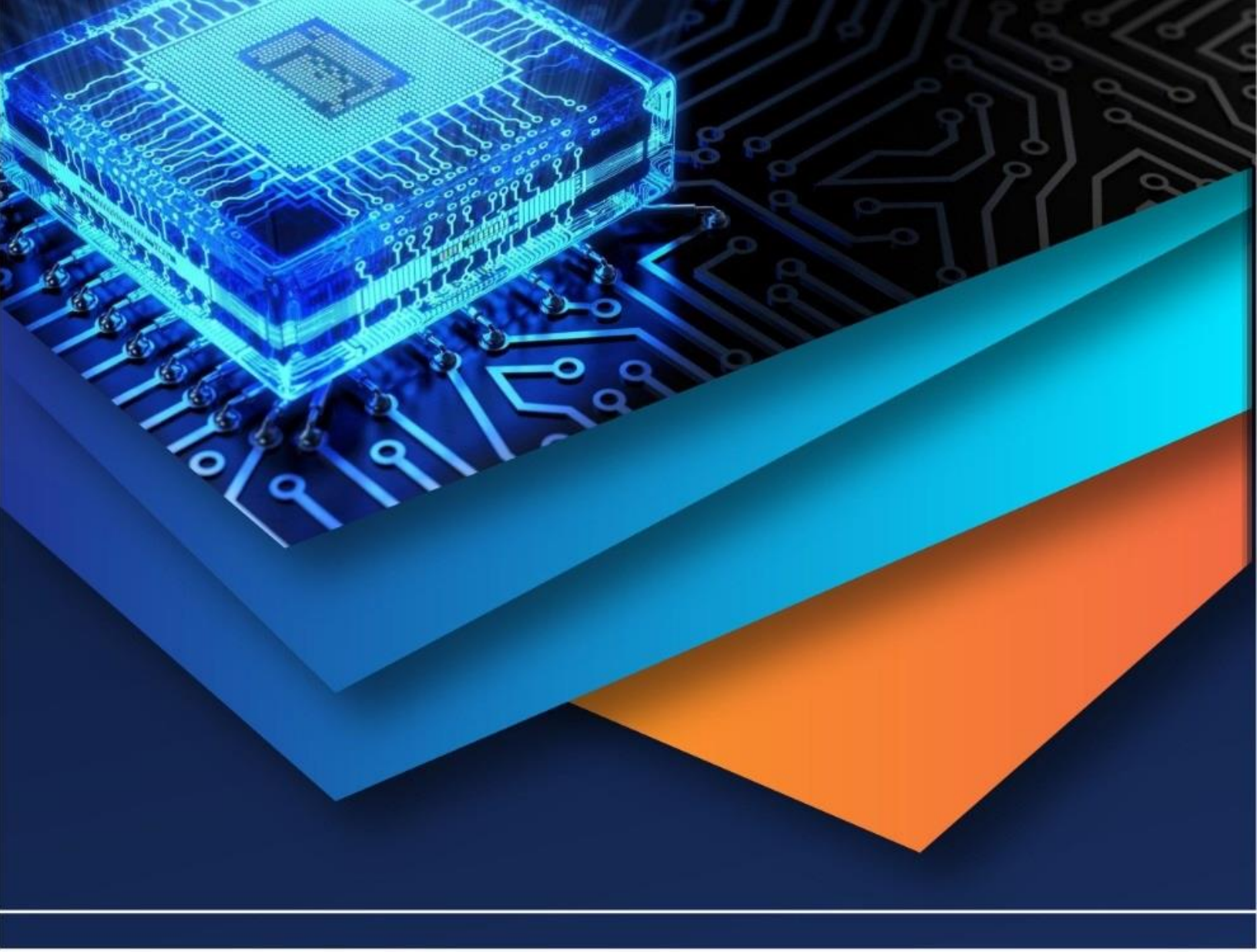

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