REVIEW

Medical Journal of Western Black Sea
Batı Karadeniz Tıp Dergisi

Med J West Black Sea 2025;9 (1): 1-6
DOI: 10.29058/mjwbs.1562437

The Effect of the Human Papilloma Virus on the Nuclei and Cellular Structure of Cervical Epithelial Cells; Light Microscopic and Cytomorphometric Analysis

Human Papilloma Virüsünün Servikal Epitel Hücrelerinin Çekirdekleri ve Hücresel Yapısı Üzerindeki Etkisi; Işık Mikroskobik ve Sitomorfometrik Analiz

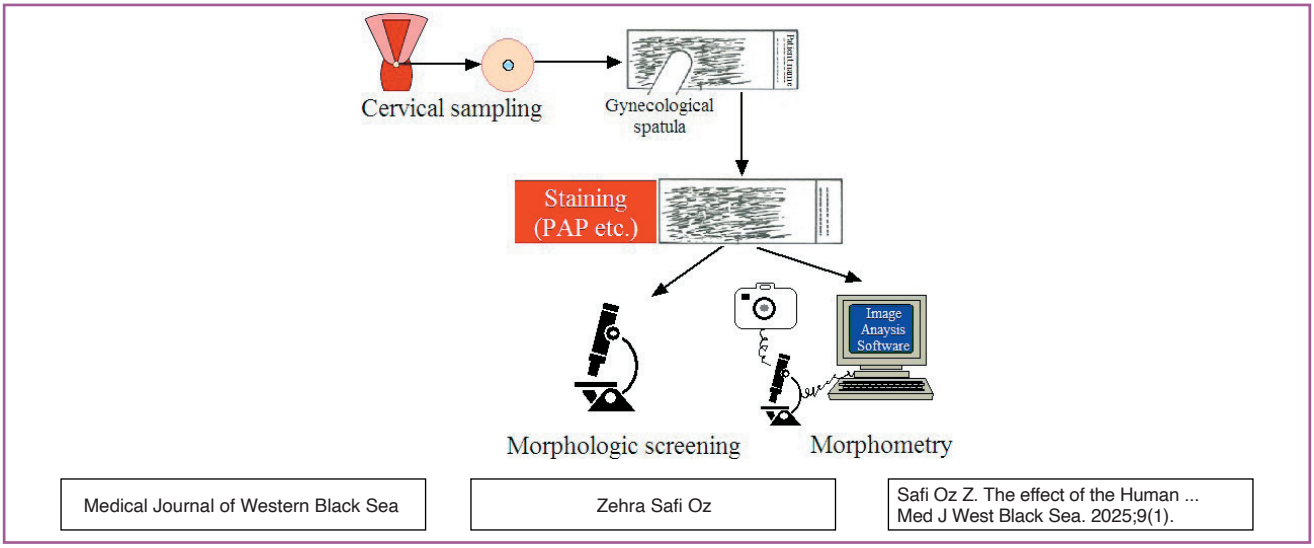
Zehra SAFI OZ

Zonguldak Bulent Ecevit University, Faculty of Medicine, Department of Medical Biology, Zonguldak, Türkiye

ORCID ID: *Zehra Safi Oz 0000-0003-1359-5026*

Cite this article as: *Safi Oz Z. The effect of the Human papilloma virus on the nuclei and cellular structure of cervical epithelial cells; light microscopic and cytomorphometric analysis. Med J West Black Sea. 2025;9(1):1-6.*

GRAPHICAL ABSTRACT



ABSTRACT

The most common sexually transmitted virus is Human papillomavirus (HPV), which comes in more than 280 types. The basal cells of epithelial tissues are responsible for taking up the HPV virus in cases of infection and malignancy caused by the virus. HPV, especially through the E6 and E7 proteins of high-risk HPV types, can cause benign infections or malignancies by inactivating tumor suppressor genes, disrupting cell cycle control, activating telomerase, preventing cell adhesion, polarity and differentiation, suppressing the immune system, and preventing apoptosis by inactivating tumor suppressor genes (especially p53, pRb).

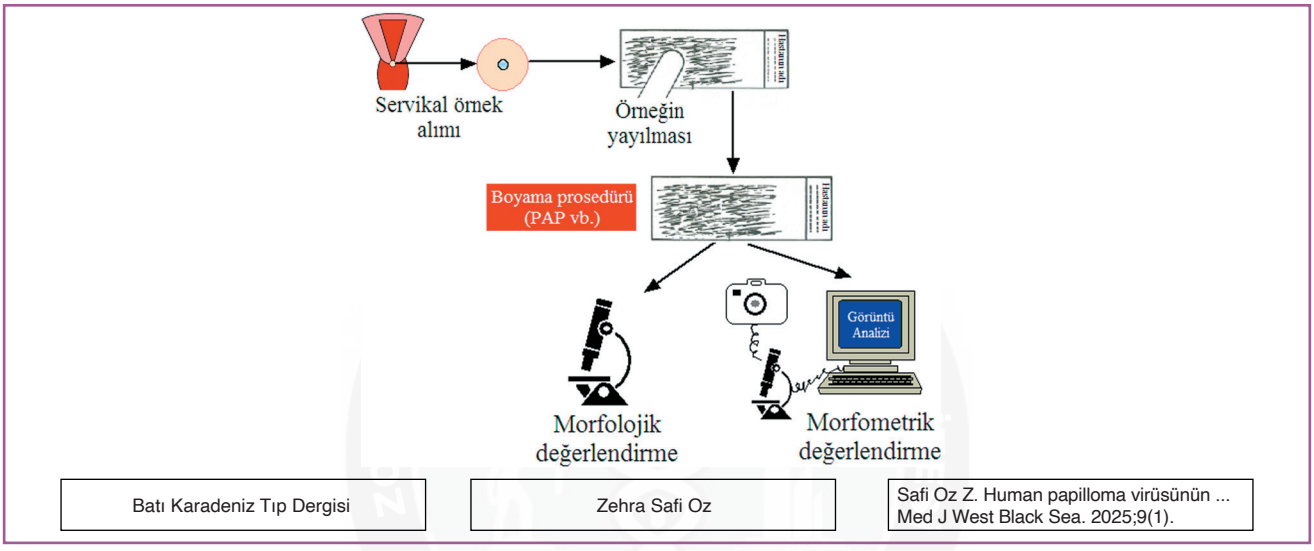
Corresponding Author: Zehra Safi Oz ✉ zehra.soz@beun.edu.tr **Received:** 06.10.2024 **Revision:** 01.04.2025 **Accepted:** 14.04.2025

In this review, the effects of Human papillomavirus on epithelial cells were evaluated in terms of morphological changes and the nucleus-cytoplasm ratio mediated by specific proteins of the virus. Morphological changes are discussed under the headings of koilocytosis, cytoplasmic vacuolization, nuclear enlargement, nuclear membrane irregularities, hyperchromasia, abnormal chromatin distribution, micronucleation, binucleation, karyorrhexis, karyolysis karyopyknosis and nuclear budding. Nuclear architecture changes, which play a crucial role in gene regulation, are particularly prominent.

Finally, it is believed that knowledge of the changes that HPV causes in cell nuclear and cytoplasmic texture will facilitate the development of a cost-effective and appropriate approach to explaining cervical cancer pathophysiology, the development of specific biomarkers, and the development of new perspectives in understanding HPV's impact on cancer.

Keywords: Human Papilloma virus, HPV, cervical cancer, cytology, Squamous epithelial cell, PAP smear

GRAFİKSEL ÖZET



ÖZ

İnsan Papilloma Virüs (HPV), cinsel yolla bulaşan en yaygın viral enfeksiyon etkenidir ve 280'den fazla tipi bulunmaktadır. HPV'nin neden olduğu enfeksiyon ve malignitede virüsün epitel dokunun bazal hücrelerince alınması gerekmektedir. HPV özelllikle de yüksek riskli HPV tiplerinin E6 ve E7 proteinleri aracılığıyla, tümör baskılayıcı genlerin (özellikle p53, pRb) inaktivasyonu, hücre döngüsü kontrolünün bozulması, telomerazın aktivasyonu, hücre adezyonu, polarite ve epitel hücre farklılaşmasının bozulması, immün sistemin baskılanması ve apoptozun engellenmesi ile benign enfeksiyonlara ya da maligniteye neden olabilir.

Bu derlemede, Human papillomavirüsün epitel hücre üzerindeki etkileri virüsün özel proteinleri aracılığıyla gerçekleşen morfolojik değişiklikler ve nükleus-sitoplazma oranı açısından değerlendirilmiştir. Morfolojik değişiklikler, koilositozis, sitoplazmik vakuolizasyon, nükleer genişleme, nükleer membran düzensizlikleri, hiperkromazi, anormal kromatin dağılımı, mikronükleasyon, binükleasyon, karyoreksis, karyoliz karyopiknozis ve nükleer tomurcuklanma başlıkları altında ele alınmıştır. Gen regülasyonunda önemli rol oynayan nükleer mimarideki değişiklikler özelllikle öne çıkmaktadır. Sonuç olarak, HPV'nin hücrenin nükleer ve sitoplazmik tekstüründeki değişikliklerinin bilinmesinin, servikal karsinogenez patofizyolojisini açıklamak için uygun ve maliyet-etkin bir yaklaşımın önerilmesine, HPV kanserleri için spesifik biyobelirteçlerin geliştirilmesine ve HPV'nin karsinogenezdeki etkilerini anlamada yeni bakış açılarının geliştirilmesine katkıda bulunacağı düşünülmektedir

Anahtar Sözcükler: İnsan papilloma virus, İPV, serviks kanseri, sitoloji, skuamöz epithel hücre, PAP simir

INTRODUCTION

Human papilloma virus (HPV) consists of circular, covalently closed double-stranded DNA and a non-enveloped icosahedral capsid. To date, over 280 HPV genotypes have been identified based on differences in the HPV DNA sequence. Viral particles consist of eight open reading frames. The HPV genome is divided into three sections: the early (E) gene region (E1, E2, E4, E5, E6 and E7), the late gene region (L1, L2) and the long control region (LCR). LCR regulates the origin of replication (1). This virus infects basal cells and its active cycle is associated with cellular differentiation. Through its structural proteins, HPV causes various alterations. These cellular effects are presented under the headings of morphology and morphometry below (2-11).

EFFECTS OF HPV ON MORPHOLOGY

In HPV infection, koilocytosis is the most prominent cellular change in differentiated squamous epithelial strata. This is formed as a result of the interaction of cytoskeletal elements especially actin and microtubules and viral proteins such as E6 (HPV16, 18), E7 (HPV16, 18, 38), E5 (high and low-risk HPV types), E1^E4 (HPV16), E4 (HPV 16) (3). Cytoplasmic vacuolization is a morphological change observed in the epithelial cell cytoplasm due to various pathogens, including HPV (12).

Many significant degenerative nuclear alterations such as membrane irregularities, nuclear enlargement, hyperchromasia, abnormal chromatin distribution, micronucleation (MN) binucleation (BN), karyorrhexis (KR), karyolysis (KL), karyopyknosis (KP) and nuclear budding (NB) induced by HPV are detectable by light microscopy in Papanicolaou Smear (Figure 1). HPV leads to disarrangement of the nuclear lamina. This is responsible for maintaining nucleus morphology and is also an attachment point for chromosomes. Disruption of the lamina causes chromatin organization, gene expression and chromosomal instability irregularities. It can also cause nuclear membrane irregularities (4,13). Micronuclei (MN) indicate chromosomal instability (14-17). It is often a broken chromosome fragment (16). Previous studies have shown that micronuclei were significantly higher in the HPV 16/18 group. (10, 18, 19). However, it is still unclear how HPV affects micronucleus formation. HPV proteins support malignant cell proliferation by disrupting p53 of E6 and pRb of E7. Evaluation of MN is valuable for monitoring HPV-induced chromosomal instability. In carcinogenesis, HPV affects many significant mechanisms such as cell cycle, apoptosis, and tumor growth suppression (10). As a result of this interaction, karyorhexis, karyolysis, and karyopyknosis occur in cells. Karyorhexis is a nuclear membrane fragmentation. Chromatin breaks into small basophilic granules and spreads into the cytoplasm in this process. Karyolysis is a cell nucleus dissolution. Karyopyknosis involves shrinkage or condensation of the cell due to increased nucleus compactness. Although these changes are specific to infection, it remains unknown at what level these degenerative changes occur in that infection (20). Exfoliated epithelial cells undergo binucleation as a reactive cellular change (21). Although the mechanism of binucleation formation in HPV infections has not been explained in detail yet, it is thought that HPV structural proteins interact with cytoskeletal elements and affect cytokinesis, so cytoplasm division does not occur following nuclear division. Nuclear budding (NB) is commonly seen in cancer and is associated with chromosomal instability. NBs are connected to the nucleus by stalks of nucleoplasmic material depending on the stage of the budding process (13, 22). In addition to these changes, HPV causes nuclear enlargement and chromatin texture changes. Nuclear enlargement is one of the characteristic alterations in malignant cells. In these cells, nuclear enlargement is thought to produce abnormal nuclear shapes to maintain the ratio of nucleus to cytoplasm. Normal cells tend to maintain the ratio of the nucleus volume to cell volume. Along with the HPV-induced change in gene expression, the change in chromatin organization may also lead to nuclear architecture change (10, 23, 24). Alterations in nuclear architecture and chromatin organization during carcinogenesis and tumor progression can also lead to nuclear shape changes in the nucleus (24). Epigenetic modulations in chromatin and the nuclear membrane are related to gene expression and carcinogenesis (23, 25).

In the literature, Guillaud et al. indicate that a correlation between HPV and chromatin condensation and measuring E2 and E6/E7 expression will effectively reveal this relationship (26). In addition, Gautam and Moody indicated that DNA damage response (DDR) facilitates HPV replication (27). Understanding how DDR alters viral chromatin modifications in HPV infection is a crucial question to clarify in the future.

EFFECTS OF HPV ON MORPHOMETRY

Computerized image analysis is one of the methods used to eliminate diagnostic variability in cytological and histopathological samples, with high sensitivity and reproducibility. Digital morphometry allows observers to create a database of various parameters to characterize cells as normal, preneoplastic, or neoplastic (28,29). Digital morphometry also has reproducibility, save digital records, and reuse them when necessary. Morphometry also characterizes cells of size, area, and shape (10, 23, 30). This method can provide two-dimensional measurements of parameters such as cellular area (CA) and nuclear area (NA), perimeter, and diameter, and can additionally be used to evaluate the staining

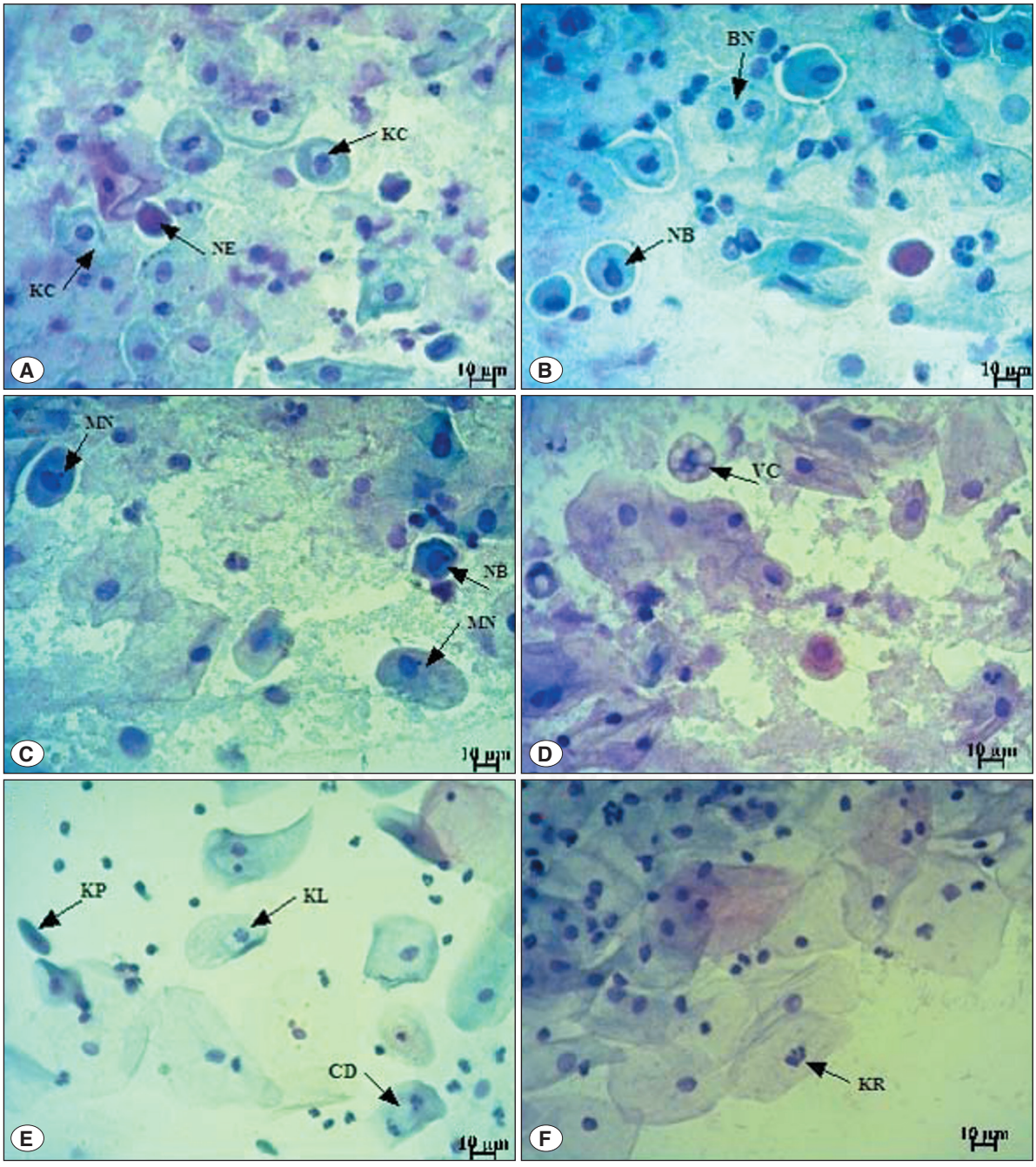


Figure 1. General appearance of cervical epithelial cells in HPV infection (Papanicoaou X 40). **A)** Exfoliated cervicovaginal epithelial cells with koilocytosis (KC) and nuclear enlargement (NE). **B)** Binucleated epithelial cell (BN) and nuclear budding (NB). **C)** Micronucleated (MN) cells and nuclear budding (NB). **D)** Vacuolization in cervical epithelial cell (VC). **E)** Karyolytic (KL) and karyopynotic (KP) cells and cellular division (CD). **F)** Karyorhectic epithelial cell (KR)

intensity of the cell and nucleus. Furthermore, it can detect subtle changes in cell shape, size, and/or texture, which can't be seen with light microscopy or even electron microscopy (28-31).

HPV causes various changes in the cell nucleus and cytoplasm, and these changes can be observed microscopically in cytological smears (Figure 1). These changes are detailed under the title "Effects of HPV on morphology". Cellular changes in HPV are also reflected in the morphometry of the cells (Figure 2). Evaluation of NA, CA and NA/CA provides non-classical HPV cytological data. These non-classical criteria can be evaluated by morphometric methods. Morphometry can provide information not detected by tradi-

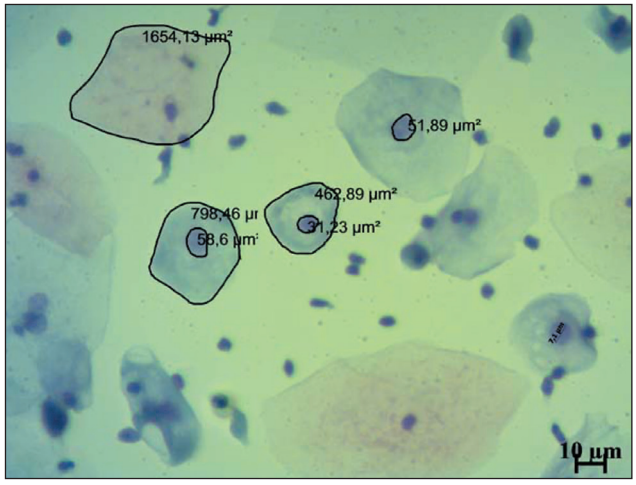


Figure 2. Measuring cellular diameter of Papanicolaou stained cervical epithelial cells in HPV infection.

tional cytology. It can explain the incompatibilities between molecular methods and morphological approaches.

There are limited studies in the literature on the morphometric changes of HPV. Safi Oz et al. measured cellular, nuclear, and cytoplasmic areas in their cytomorphometric study of patients infected with HPV 16. They calculated the nucleus/cytoplasmic ratio and compared it with the control group. Cellular, nuclear, and cytoplasmic areas were lower in the HPV 16 positive group than the control group. However, no statistically significant difference was found between the values (10).

Safi Oz et al., in their cytomorphometric study of HPV 18 infected patients, measured the cellular, nuclear and cytoplasmic area. They calculated the nucleus/cytoplasmic ratio, and measured the width, length and perimeter values of the cell and nucleus. They compared them with the control group. Cellular area, nuclear area, cytoplasmic area, nucleus/cytoplasm ratios, cell width, length and perimeter values and nucleus width, length and perimeter values were lower in the HPV 18 positive group than in the control group. The nucleus width and length values were also statistically significant between the HPV 18 and control groups (31). It ıs consıdered that morphologıcal and morphometrıc studİes related to HPV will contribute to the enlightenment of the mechanism of HPV infection.

CONCLUSION

This review evaluated HPV structural effects on cervical cell morphology and morphometry. Knowing the structural changes induced by HPV can help gain new perspectives on gene regulation architecture in the cell. This will enable us to understand HPV's effects on carcinogenesis. Micronucleus scoring may contribute to the evaluation of different HPV types in this respect, as it shows chromosomal instability in this infection. Since the texture of the nucleus shows the degree of chromatin condensation and integration of HPV, a detailed examination of the texture in the presence of different HPV types is thought to be a valuable and effective tool in cervical cell evaluation and is potentially useful for the development of specific drug targeting and biomarkers specific to HPV-induced cancer. Morphometry is thought to provide information that cannot be detected by traditional cytological methods. It is also thought to explain the incompatibilities between molecular methods and morphological approaches. I believe that morphometric evaluations need to be performed on different HPV types.

Acknowledgements

None.

Author Contributions

The study conception and design, writing of the manuscript, and preparation of images were all made by the author.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Financial Support

This research received no external funding.

Ethical Approval

Since it is not an experimental or human study and is a review, ethical approval is not required. In the preparation process of this study, scientific and ethical principles were followed and all studies used were indicated in the references. This article was scanned by iTenticate software. The plagiarism rate was detected as 14%.

Review Process

Extremely and externally peer-reviewed.

REFERENCES

1. Boda D, Docea A, Calina D, Ilie M, Caruntu C, Zurac S, Neagu M, Constantin C, Branisteanu D, Voiculescu V, et al. Human papilloma virus: Apprehending the link with carcinogenesis and unveiling new research avenues (Review). International Journal of Oncology (2018) doi: 10.3892/ijo.2018.4256
2. Howie HL, Katzenellenbogen RA, Galloway DA. Papillomavirus E6 proteins. Virology 2009;384:324–334. doi: 10.1016/j.virol.2008.11.017
3. Safi Oz Z. The ınteraction between human papillomavirus proteins and cytoskeletal filaments. (2012). In: Human Papillomavirus and Related Diseases - From Bench to Bedside - Research aspects / Book title: ISBN 978-953-307-855-7 Editor: Dr. Davy Vanden Broeck; Intech -Open Access Publisher. Hard cover, 406 pages, 291- 310 http://www.intechopen.com/articles/show/title/the-interact-on-between-human-papillomavirus-proteins-and-cytoskeletal-filaments. DOI: 10.5772/28745

4. Smith ER, George SH, Kobetz E, Xu X. New biological research and understanding of Papanicolaou's test. Diagnostic Cytopathology 2018; 46:507–515. doi: 10.1002/dc.23941
5. Gheit T. Mucosal and cutaneous human papillomavirus ınfections and cancer biology. Frontiers in Oncology 2019; 8;9:355. doi: 10.3389/fonc.2019.00355
6. Kwon EJ, Ha M, Jang JY, Kim YH. Identification and complete validation of prognostic gene signatures for human papillomavirus-associated cancers: Integrated approach covering different anatomical locations. Journal of Virology 2021;24;95(6):e02354-20. doi: 10.1128/jvi.02354-20
7. Della Fera AN, Warburton A, Coursey TL, Khurana S, McBride AA. Persistent human papillomavirus ınfection. Viruses 2021;13:321. doi: 10.3390/v13020321
8. Díaz-Tejeda Y, Guido-Jiménez MC, López-Carbajal H, Amador-Molina A, Méndez-Martínez R, Gariglio-Vidal P, Lizano M, García-Carrancá A. Nanog, in cooperation with AP1, ıncreases the expression of E6/E7 oncogenes from HPV types 16/18. Viruses 2021;28;13(8):1482 doi: 10.3390/v13081482
9. Mattoscio D, Gheit T, Strati K, Venuti A. Editorial: HPV and host ınteraction. Frontiers in Cellular and Infection Microbiology 2021;11:11:638005. doi: 10.3389/fcimb.2021.638005
10. Safi Oz Z, Onak Kandemir N, Kokturk F, Ozdamar SO. Cytomorphometry and morphology analysis of human papillomavirus type 16 in liquid-based cervical cytology samples. Diagnostic Cytopathology 2021;49:885–893. doi: 10.1002/dc.24761
11. Warowicka A, Broniarczyk J, Węglewska M, Kwaśniewski W, Goździcka-Józefiak A. Dual role of YY1 in HPV life cycle and cervical cancer development. International Journal of Molecular Sciences 2022;23:3453. doi: 10.3390/ijms23073453
12. Shubin AV, Demidyuk IV, Komissarov AA, Rafieva LM, Kostrov SV. Cytoplasmic vacuolization in cell death and survival. Oncotarget 2016;7:55863–55889. doi: 10.18632/oncotarget.10150
13. Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Norppa H, Eastmond DA, Tucker JD, Thomas P. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 2010;26:125–132. doi: 10.1093/mutage/geq052
14. Safi Oz Z, Dogan Gun B, Ozdamar SO. Evaluation of micronuclei, nuclear anomalies and the nuclear/cytoplasmic ratio of exfoliated cervical epithelial cells in genital candidiasis. Acta Cytologica 2015;59:180–186. doi: 10.1159/000381615
15. Safi Oz Z, Doğan Gun B, Ozdamar SO. Why an experienced eye may not detect micronucleated cells using papanicolaou's stain. Acta Cytologica 2015;59:431–433. doi: 10.1159/000440934
16. Safi Oz Z. Micronucleus scoring: An available approach in the evaluation of genomic damage in exfoliative cervicovaginal cells. Annals of Cytology and Pathology 2020; 5:064–067. doi: 10.17352/acp.000018
17. Nersesyan A, Muradyan R, Kundi M, Fenech M, Bolognesi C, Knasmueller S. Smoking causes induction of micronuclei and other nuclear anomalies in cervical cells. International Journal of Hygiene and Environmental Health 2020;226:113492. doi: 10.1016/j.ijheh.2020.113492
18. Sancer O, Şirin MC, Arıdoğan B, Sesli Çetin E, Yiğit A, Tepebaşı MY, Aslan Koşar P. Comparison of DNA damage and proliferative capacities in smear samples of HPV positive and negative patients by micronucleus counting and AgNOR staining. Biotechnic & Histochemistry 2020;96:354–358. doi: 10.1080/10520295.2020.1800091
19. Shi Y-H, Wang B-W, Tuokan T, Li Q-Z, Zhang Y-J. Association between micronucleus frequency and cervical intraepithelial neoplasia grade in Thinprep cytological test and its significance. International journal of clinical and experimental pathology 2015;8:8426–32.
20. Peter Vooijs G, van Aspert-van Erp AJM, Bulten J. "Benign proliferative reactions, ıntraepithelial neoplasia, and ınvasive cancer of the uterine cervix.," Comprehensive Cytopathology. Elsevier (2008). p. 131–212 http://dx.doi.org/10.1016/b978-141604208-2.10008-9 [Accessed September 1, 2024]
21. Okodo M, Okayama K, Fukui T, Shiina N, Caniz T, Yabusaki H, Fujii M. Significance of Compression in Binucleation while Differentiating Reactive Cellular Changes Between Human Papillomavirus and Candida Infections. Asian Pacific Journal of Cancer Prevention 2017;18:2507–2511. doi: 10.22034/APJCP.2017.18.9.2507
22. Mitkovska VI, Dimitrov HA, Chassovnikarova TG. nduction of Erythrocytic Nuclear Abnormalities by Permitted Concentration of Cadmium in Common Carp (Cyprinus carpio L.). Ecologia balkanica 2018;10:235–247.
23. Dey P. Cancer nucleus: Morphology and beyond. Diagnostic Cytopathology 2010;38:382–390. doi: 10.1002/dc.21234
24. Fischer EG. Nuclear morphology and the biology of cancer cells. Acta Cytologica 2020; 64:511–519. doi: 10.1159/000508780
25. Da Silva M, De Albuquerque B, Allyrio T, De Almeida V, Cobucci R, Bezerra F, Andrade V, Lanza D, De Azevedo J, De Araújo J, et al. The role of HPV-induced epigenetic changes in cervical carcinogenesis (Review). Biomedical Reports 2021;15(1):60. doi: 10.3892/br.2021.1436
26. Guillaud M, Adler-Storthz K, Malpica A, Staerkel G, Matisic J, Van Niekirk D, Cox D, Poulin N, Follen M, MacAulay C. Subvisual chromatin changes in cervical epithelium measured by texture image analysis and correlated with HPV. Gynecologic Oncology 2005;99:S16–S23. doi: 10.1016/j.ygyno.2005.07.037
27. Gautam D, Moody CA. Impact of the DNA damage response on human papillomavirus chromatin. PLOS Pathogens 2016;16;12(6):e1005613. doi:10.1371/journal.ppat.1005613
28. Sana, DEM, Mayrink de Miranda,P, Pitol, BCV, Moran, MS, Silva NNT, Guerreiro da Silv, IDC, Carneiro CM. Morphometric evaluation and nonclassical criteria for the diagnosis of HPV infection and cytological atypia in cervical samples. Diagnostic Cytopathology, 2013: 41(9), 785–792. doi:10.1002/dc.22955
29. Kafa İM, Arı İ. The Comparison of Manuel and Digital Metric Measurement Methods in Morphometric Studies.Journal of Uludağ University Medical Faculty September 2004;30(3):141-144.
30. Scheurer ME, Guillaud M, Tortolero-Luna G, McAulay C, Follen M, Adler-Storthz K. Human papillomavirus-related cellular changes measured by cytometric analysis of DNA ploidy and chromatin texture. Cytometry Part B: Clinical Cytometry 2007;72B:324–331. doi: 10.1002/cyto.b.20173
31. Safi Oz Z, Barut F, Kokturk F, Gun Bd. The structural profile of HPV 18 in terms of chromosomal and nuclear degenerative changes and the ratio of nucleus/cytoplasm on liquid based cervical cells. J Cytol 2023;40:133-9.