ORIGINAL RESEARCH

Comparative analysis of stromal inflammatory cell infiltration and HPV infection status in cervical dysplasia and squamous cell carcinoma using the Klintrup-Mäkinen Scoring method

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ABSTRACT

Objective: Cervical cancer is the second most common malignancy in women, with 604,127 new cases reported globally in 2020. Persistent high-risk human papillomavirus (HPV) infection is the primary cause. This study aimed to evaluate the relationship between stromal inflammatory cell infiltration using the Klintrup-Mäkinen (KM) score and HPV infection status in cervical dysplasia and squamous cell carcinoma (SCC).

Materials and Methods: A cross-sectional study was conducted using secondary data from formalin-fixed paraffin-embedded (FFPE) cervical tissue samples diagnosed as dysplasia or SCC at Atma Jaya Catholic University of Indonesia from 2014 to 2022. HPV status was determined using PCR-based assays, while stromal inflammatory cell infiltration was assessed microscopically at 100× magnification and graded according to the KM scoring system by two blinded observers. Statistical analyses, including Chi-square and Spearman's correlation tests, were performed using STATA/IC 15, with $p < 0.05$ considered significant.

Results: A total of 38 samples were analyzed, comprising 20 (52.63%) cases of dysplasia and 18 (47.37%) cases of SCC. HPV positivity was identified in 70% of dysplasia and 94.44% of SCC cases. KM scores of 2/3, indicating moderate to severe inflammatory infiltration, were significantly more frequent in SCC (77.78%) compared to dysplasia (20%), showing a strong association with pathological diagnosis ($p = 0.0019$). However, no significant correlation was observed between KM score and HPV status in either lesion type.

Conclusion: The KM scoring method provides a reliable and cost-effective approach for assessing stromal inflammatory cell infiltration in cervical lesions, aiding histopathological diagnosis, although it does not predict HPV infection status.

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Highlights:

1. KM score is a reliable method to help determine the diagnosis of cervical dysplasia and squamous cell carcinoma.
2. KM score cannot be used to differentiate infection status in cervical dysplasia and squamous cell carcinoma.

INTRODUCTION

Cervical cancer is a prevalent neoplasm among women. According to the Global Cancer Observatory (GLOBOCAN),[1] cervical cancer ranks as the second most common cancer in women after breast cancer, with an estimated 604,127 new cases worldwide in 2020, 90% of which occur in low- to middle-income populations. According to data from the HPV Information Centre ICO/IARC, cervical cases in Indonesia accounted for an estimated 36,633 cases in 2020, making it the second most common cancer in Indonesian women,[2] The primary cause of cervical cancer is infection with high-risk Human Papilloma Virus (hr-HPV).[3]

A study by Xie et al.[4] identified several factors that can be used to determine prognostic value, including FIGO classification, age, recurrence, and comorbidities. According to Zou et al.,[5] inflammatory cell infiltration in the stromal tissue of cancer can serve as a prognostic indicator for cervical cancer patients, although this remains debatable. Previous studies[6,7,8] on other cancers (such as breast cancer and colorectal cancer) have also shown a correlation between stromal inflammatory cell infiltration and prognosis. For instance, studies by Fortis et al.,[6] Kim et al.,[7] and Väyrynen et al.[8] revealed a significant association between inflammatory cell infiltration and patient outcomes, such as recurrence rate, disease-free survival, and overall survival. Additionally, Evans et al.[9] reported that cervical cancer caused by HPV exhibits a higher level of inflammatory cell infiltration compared to non-HPV-induced cervical cancer. However, assessing inflammatory cell infiltration in cancer stroma typically requires immunohistochemical (IHC) staining, which can be prohibitively expensive.[10] This poses a significant challenge, particularly since the majority of cervical cancer cases occur in low- to middle-income communities.

Bray et al.[11] further highlighted that socioeconomic factors influence disparities in cervical cancer prevalence. In high-income countries, improved access to screening facilitates early detection and reduces mortality rates. There is a pressing need for a more cost-effective and accurate staining method to address these challenges. The Klintrup-Mäkinen (KM) score, a semi-quantitative method, has shown promising results in evaluating inflammatory cell infiltration in the stroma of colorectal cancer.[12] This method utilizes routine hematoxylin and eosin (H&E) staining and adds no additional costs. Given the abovementioned issues, this study aims to determine whether the semi-quantitative KM method using H&E staining can be a viable alternative for assessing prognostic indicators through inflammatory cell infiltration in cervical cancer stroma.

MATERIALS AND METHODS

Methods

This was a cross-sectional study in which pathological diagnosis data were collected from medical records at the Department of Anatomical Pathology, School of Medicine and Health Science Atma Jaya Catholic University of Indonesia, covering the period from 2014 to 2023. Ethical clearance was obtained under approval number 28/02/KEP-FKIKUAJ/2023, issued on February 20, 2023, by the Research Ethics Committee of Atma Jaya Catholic University of Indonesia. Cervical tissue specimens were obtained through biopsy or surgical procedures, fixed in 10% neutral-buffered formaldehyde (NBF), and embedded in paraffin. HPV infection status (positive or negative) for each sample was determined using PCR testing, DNA hybridization and multiplex PCR techniques. If any of the test results showed a positive HPV status, the case was recorded as HPV-positive.

Inclusion and exclusion criteria

All samples were cervical squamous cell carcinoma (SCC) or cervical dysplasia tissues collected between 2014 and 2023. To be included in this study, each sample was required to have sufficient DNA yield (> 5 ng/µL) and satisfactory DNA purity (A260/A280 ratio > 1.5). Samples that could not be retrieved or were significantly damaged were excluded from this study.

Klintrup-Mäkinen score analysis

Slides obtained from the Department of Anatomical Pathology, School of Medicine and Health Science, Atma Jaya Catholic University of Indonesia, were analyzed under a microscope at 100× magnification in the area with the highest inflammatory cell infiltration. Then, the slides were the graded according to the KM scoring system, as published by Klintrup et al., by two

blinded researcher: a researcher (AF) and a pathologist (LL)). KM score one is defined as mild stromal inflammatory cell infiltration, characterized by patchy distribution of inflammatory cells (Figure 1[1]). KM score 2 represents moderate infiltration, with a band-like arrangement of inflammatory cells along the invasive margin (Figure 1[2]). KM score 3 indicates severe stromal inflammatory infiltration, forming a cup-like pattern encircling the tumor nest (Figure 1[3]).

Extraction, amplification, and detection of nucleic acid

HPV detection was conducted using the 14 High-Risk HPV with 16/18 Genotyping Real-Time PCR Kit (GuangDong HybriBio Biotech Co. Ltd., Hong Kong). Genomic DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) cervical tissue samples using the Quick-DNA/RNA FFPE Extraction Kit (Zymo Research, California, USA), following the manufacturer's protocol. DNA concentration and purity were evaluated using the NanoDrop™ One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). The CFX-1000 Touch Real-Time PCR Detection System (Bio-Rad, California, USA) performed real-time PCR amplification and detection. Data analysis, including cycle threshold (Ct) value determination and sigmoidal curve validation, was done using the Bio-Rad CFX Maestro software (Version 4.1.2433.1219).

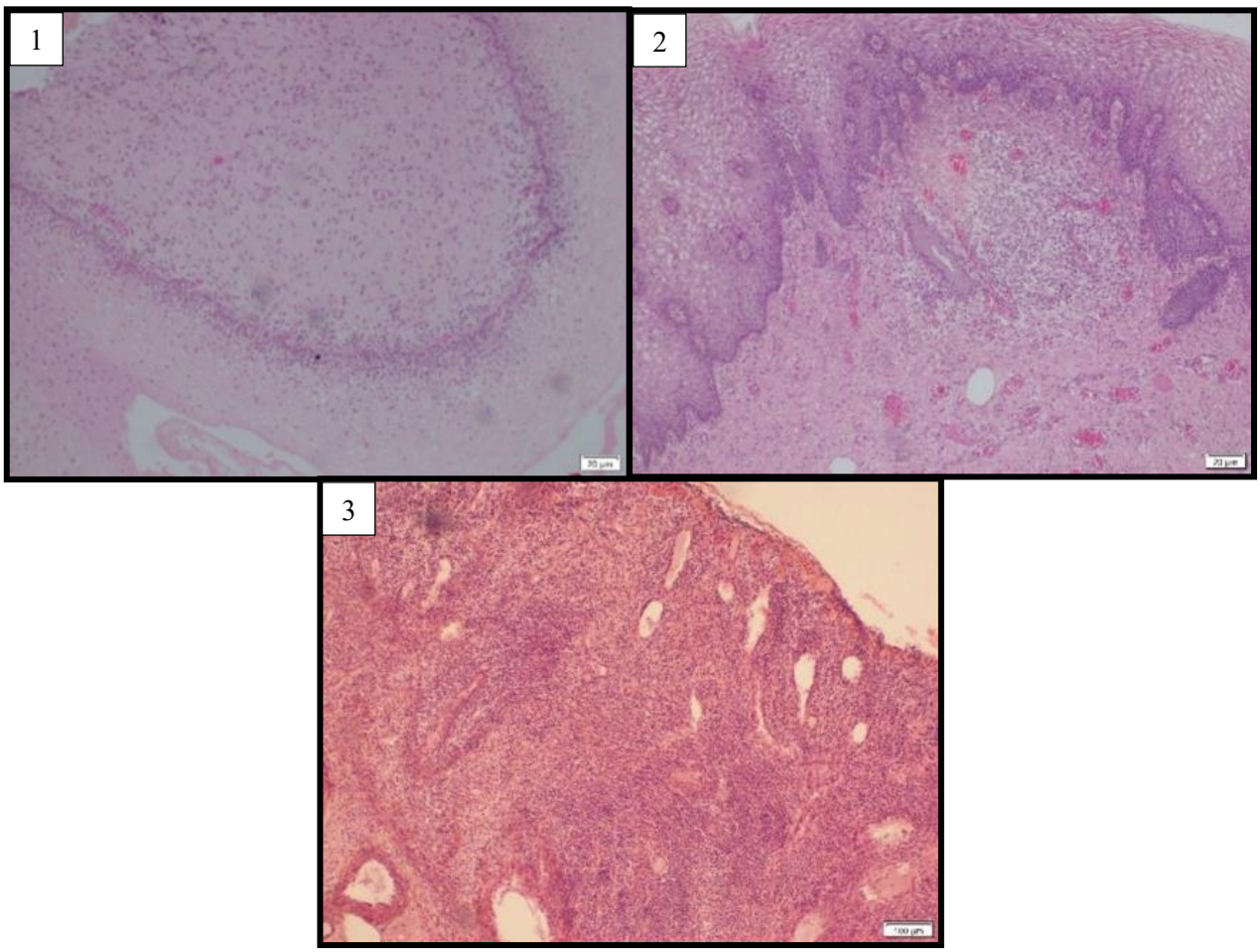

Figure 1. Stromal inflammatory cell infiltration showing (1) KM score 1; (2) KM score 2; (3) KM score 3

Data analysis

The KM score data obtained in this study were analyzed using inter-observer agreement principles. The final KM score assigned was based on the assessment of the pathologist. Data cleaning and statistical analyses were performed using STATA/IC 15 (StataCorp, College Station, TX, USA). The Cohen's Kappa coefficient was evaluated for inter-observer consistency between the researcher and pathologists. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Thirty-eight samples were included in the analysis, and their characteristics are summarized in Table 1. All samples were evaluated using the KM scoring system by two independent observers. Inter-observer agreement yielded a Cohen's Kappa value of 97.37, indicating an almost perfect agreement between the raters, as shown in Table 2. This high Kappa value demonstrates the strong reliability of the KM scoring system in assessing stromal inflammatory cell infiltration in both dysplastic and cancerous cervical tissue.

Subsequently, KM score data and pathological diagnoses were analyzed to assess their correlation using the Spearman ranks correlation test and Chi-square test for associations between categorical variables. A significant association was found between KM score and pathological diagnosis (Chi-square p-value = 0.0019), with a moderate positive correlation (Spearman's r = 0.38; p-value = 0.0179), as presented in Table 2. However, no significant association or correlation was observed between KM score and HPV infection status in cervical dysplasia or cervical cancer tissues (p > 0.05), as shown in Table 3.

Table 1. Study sample distribution

Sample Included in this study: 38 samples	Number of samples (n; %)
Sample ages (years)	
9	3; 7.89
8	8; 21.05
7	9; 23.68
6	7; 18.42
5	5;13.16
4	6; 15.79
Pathology diagnosis	
Cervical dysplasia	20; 52.63
Cervical SCC	18; 47.37
HPV genotype	
16	26; 68.42
18	9; 23.68
Others	20; 47.37
Negative	7; 18.42
KM score	
1	16; 42.11
2	12; 31.58
3	10; 26.32

Table 2. Cohen's Kappa

		Rater 1	
		1	2/3
Rater 2	1	15	0
	2/3	1	22
Kappa: 97.37%			

Table 3. Association and correlation between stromal inflammatory cell infiltration and pathological diagnosis

	Pathological diagnosis		Total (n; %)	r (P-value)	P-value
	Cervical dysplasia (n; %)	Cervical SCC (n; %)			
1	12; 31.58	4; 10.53	16; 42.11	0.382 (0.0179)#	0.0019*
2/3	8; 21.05	14; 36.84	22; 57.89		
total	20; 52.63	18; 47.37	38; 100.00		

*chi-square
#Spearman rank correlation test

Based on the results, the KM score is moderately correlated and significantly associated with the pathological diagnosis of cervical dysplasia or SCC. Cervical SCC was more likely to have a KM score of 2/3, and cervical dysplasia was more likely to have a KM score of 1. This can happen because 95% of cervical cancer samples that have been tested for HPV genetic material show a positive result. This is in concordance with the study conducted by Evans et al.,[9] which found that in HPV infected cervical cancer is associated with a higher inflammation process mediated by the introduction of higher foreign antigens.

Table 4. Association between stromal inflammatory cell infiltration and HPV infection status

	KM score		Total (n; %)	r (P-value)	P-value
	1 (n; %)	2/3 (n; %)			
Cervical dysplasia					
HPV +	10; 50.00	4; 20.00	14; 70	-0.356	0.161**
HPV -	2;10.00	4; 20.00	6; 30	(0.123)##	
Cervical cancer					
HPV +	4; 22.22	13; 72.22	17; 94.44	-0.130	1.00**
HPV-	0; 0.00	1; 5.56	1; 5.56	(0.27)##	

** Fisher's exact probability test
##Spearman's rank coefficient correlation

Furthermore, another study by Evans et al.,[13] found that HPV-positive cervical cancer shows higher expression of MHC class I and II molecules, which enhance antigen antigen presentation and subsequently promote inflammatory cell recruitment and stromal infiltration. Another factor that can cause higher stromal immune cell infiltration is histologic differences between cervical dysplasia and cancer itself, such as higher necrotic area and higher angiogenesis in cervical cancer than cervical dysplasia; these factors can cause increased immune cell response leading to higher stromal inflammatory cell infiltration.[14,15]

From the result obtained by analyzing the KM score and its association with HPV infection in dysplasia, there was no significant association and a moderately inverse correlation. Still, it is not significantly correlated with cervical dysplasia. There is a chance that this result is caused by either the sample quality or the coverage of the qPCR kit. For example, an old sample causes a decrease in sample quality; this can happen because our FFPE blocks are stored at room temperature and uncontrolled humidity levels, as in Indonesia itself, the humidity level is around 53.7-100%,[16] and there's also a chance that the qPCR kit used in this study did not cover subtypes of HPV infecting samples in this study. Besides the factors caused by the samples or qPCR kit used, there is some chance that an inverse correlation can be caused by HPV infection itself.

According to Torres-Poveda et al.[17] and Cao M et al.,[18] immunosuppression can occur in HPV-infected precancerous lesions and is directly proportional to HPV viral load. This indicates that HPV infection causes immunosuppression that promotes the development of cervical dysplasia and cervical cancer. This finding is also supported by the studies conducted by Dong et al.[19] and Mezache L et al,[20] in which there is a significant association between programmed death-ligand 1 (PD-L1) expression and HPV infection. In dysplastic cervical tissue with positive HPV infection status, PD-L1 expression increases compared with HPV-negative dysplastic lesions. Increased expression of PD-L1 can modulate and alter immune cell activity, primarily T CD8+ lymphocyte, by binding to programmed death 1 (PD-1) and forming PD L1/PD-1 complex, thus enabling the "avoiding immune surveillance" mechanism in 14 hallmarks of cancer.[21]

On the other hand, higher immune cell infiltration was observed in HPV-negative cervical dysplasia. This can happen because of several things; there is a chance these negative HPV samples might not be true HPV negative because of the old samples and degraded genetic materials or qPCR kit coverage. According to studies conducted by Shao et al.,[22] and Xie et al.,[23] increased expression of single nucleotide variant (SNV) neoantigen, mutation of the cellular gene and expression of cancer-testis agent (CTA), and insertion-deletion neoantigen in HPV negative precancerous lesion. These neoantigen potentially have some immunogenic properties that can increase local inflammatory reaction, and with increased immunogenic neoantigen production, stromal inflammatory cell infiltration will also increase. Therefore, there is also possible that the absence of HPV infection in dysplastic tissue leads to higher production of neoantigens. Clinically, these findings suggest that HPV-positive precancerous lesions may increase the likelihood of cervical dysplasia progressing to cervical cancer, and potentially resulting in worse outcomes if HPV-positive cervical dysplasia is not detected early in the disease progression.

Lastly, from the result obtained by analyzing KM score and HPV infection status in cervical SCC tissue, there's no significant correlation or association between these variables. This result is highly influenced by uneven sample distribution between the positive group and HPV negative group, in which 17 samples were HPV positive and only 1 sample was HPV negative. This finding is highly possible because, as we know about the etiopathogenesis of cervical cancer itself, where

HPV is the primary cause and risk factor for cervical cancer progression. This finding is also supported by data presented by WHO,[24] that 95% of cervical SCC across the world is caused by persistent infection of hr-HPV.

Another study by Lee et al.,[25] and Xing et al,[26] states there is a chance that 5% of the population has HPV-negative cervical cancer; this does not necessarily mean they are truly HPV-negative, with the development of newer and more sensitive methods to detect HPV genetic material, the prevalence of HPV negative cervical cancer decreases. This means there is a much smaller number of HPV-negative cervical cancer compared to HPV-positive cervical cancer than the number we know now. Because of this, the statistical analysis cannot show a valid result because the highly unbalanced sample population between 2 categories and the HPV-negative cervical SCC sample does not meet the minimum sample size for this study and thus does not have enough statistical power due to this study is an interim report and only based on a single center study.

The strength of this study was the novelty of this method in cervical cancer inflammatory cell infiltration analysis that can be used for further studies (e.g., prognosis determination, treatment effectivity). The limitation of this study was the limited sample size; some samples are outdated, which can alter the samples' quality, and the genetic material produced from qPCR. In this study, the researcher did not analyze other factors that may contribute to stromal immune cell infiltration.

CONCLUSION

There is a significant correlation and association between KM score and pathological diagnosis (cervical cancer or cervical dysplasia), in which a higher degree of pathological diagnosis also exhibits a higher degree of stromal inflammatory cell infiltration according to the KM scoring system when entirely neglecting the HPV infection status of the examined tissue. There is no significant association nor correlation between the KM score and HPV infection status either in cervical cancer or cervical dysplasia, in which, statistically, the HPV infection status does not contribute to an increased or decreased stromal inflammatory cell infiltration level. Further studies with bigger samples and other factors that affect stromal inflammatory cell infiltration must be included.

DISCLOSURES

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Conflict of interest

There is no conflict of interest with this study.

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Author contribution

NAF: Conceptualization, Methodology, Data curation, Writing - Original draft preparation. L: Conceptualization, Supervision, Validation, Visualization, Writing - Reviewing and Editing. DE: Conceptualization, Investigation, Formal Analysis, Writing - Reviewing and Editing. CA: Conceptualization, Methodology, Data curation, Writing – Reviewing and Editing.

REFERENCES

1. GLOBOCAN 2020: N1. GLOBOCAN 2020: New global cancer data. UICC [Internet]. [cited 2023 December 14]. Available from: https://www.uicc.org/news/globocan-2020-new-global-cancer-data.
2. HPV Information Center. Indonesia: Human Papillomavirus and Related Cancers. Fact Sheet 2023. Available from https://hpvcentre.net/statistics/reports/IDN_FS.pdf
3. Kumar V, Abbas AK, Aster JC. Robbins & Cotran Pathologic Basis of Disease. 10th edn. Elsevier; 2020. Chapter 22; p. 996-1000.
4. Xie L, Chu R, Wang K, et al. Prognostic assessment of cervical cancer patients by clinical staging and surgical-pathological factor: a support vector machine-based approach. Front Oncol. 2020; 10:1353. doi: 10.3389/fonc.2020.01353. PMID: 32850433; PMCID: PMC7419674.
5. Zou R, Gu R, Yu X, Hu Y, Yu J, Xue X, et al. Characteristics of infiltrating immune cells and a predictive immune model for cervical cancer. J Cancer. 2021 April 20;12(12):3501–14.
6. Fortis SP, Sofopoulos M, Sotiriadou NN, Haritos C, Vaxevanis CK, Anastasopoulou EA, et al. Differential intratumoral distributions of CD8 and CD163 immune cells as prognostic biomarkers in breast cancer. J Immunother Cancer. 2017 April 18;5:39.

7. Kim DH, Kim SW, Han JS, Kim GJ, Basurrah MA, Hwang SH. The prognostic utilities of various risk factors for laryngeal squamous cell carcinoma: A Systematic Review and Meta-Analysis. Medicina (Kaunas). 2023 Mar 2;59(3):497.
8. Väyrynen JP, Tuomisto A, Klintrup K, Mäkelä J, Karttunen TJ, Mäkinen MJ. Detailed analysis of inflammatory cell infiltration in colorectal cancer. Br J Cancer. 2013 Oct;109(7):1839–47.
9. Evans AM, Salnikov M, Gameiro SF, Maleki Vareki S, Mymryk JS. HPV-Positive and -negative cervical cancers are immunologically distinct. J Clin Med. 2022 Aug 18;11(16):4825.
10. Dunstan RW, Wharton KA, Quigley C, Lowe A. The Use of Immunohistochemistry for Biomarker Assessment—Can It Compete with Other Technologies? Toxicol Pathol. 2011 Oct;39(6):988–1002.
11. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6):394–424.
12. Klintrup K, Mäkinen JM, Kauppila S, Väre PO, Melkko J, Tuominen H, et al. Inflammation and prognosis in colorectal cancer. Eur J Cancer Oxf Engl 1990. 2005 Nov;41(17):2645–54.
13. Evans AM, Salnikov M, Tessier TM, Mymryk JS. Reduced MHC Class I and II expression in HPV?negative vs HPV?positive cervical cancers. Cells. 2022 Dec 3;11(23):3911.
14. Dutta S, Going JJ, Crumley ABC, Mohammed Z, Orange C, Edwards J, et al. The relationship between tumour necrosis, tumour proliferation, local and systemic inflammation, microvessel density and survival in patients undergoing potentially curative resection of oesophageal adenocarcinoma. Br J Cancer. 2012 Feb;106(4):702–10.
15. Carrero YN, Callejas DE, Mosquera JA. In situ immunopathological events in human cervical intraepithelial neoplasia and cervical cancer: Review. Transl Oncol. 2021 Mar 4;14(5):101058.
16. 2m (Permukaan) [Internet]. [cited 2025 January 13]. Available from: https://web-meteo.bmkg.go.id/id/model-prediksi-cuaca/nwp-parameter-cuaca/kelembapan/prediksi-kelembapan-udara-permukaan-2m
17. Torres-Poveda K, Bahena-Román M, Madrid-González C, Burguete-García AI, Bermúdez-Morales VH, Peralta-Zaragoza O, et al. Role of IL-10 and TGF-?1 in local immunosuppression in HPV-associated cervical neoplasia. World Journal of Clinical Oncology. 2014 Oct 10;5(4):753.
18. Cao M, Wang Y, Wang D, Duan Y, Hong W, Zhang N, et al. Increased high-risk human papillomavirus viral load Is associated with immunosuppressed microenvironment and predicts a worse long-term survival in cervical cancer patients. Am J Clin Pathol. 2020 March 9;153(4):502–12.
19. Dong P, Xiong Y, Yu J, Chen L, Tao T, Yi S, et al. Control of PD-L1 expression by miR-140/142/340/383 and oncogenic activation of the OCT4–miR-18a pathway in cervical cancer. Oncogene. 2018 May 31;37(39):5257.
20. Mezache L, Paniccia B, Nyinawabera A, Nuovo GJ. Enhanced expression of PD L1 in cervical intraepithelial neoplasia and cervical cancers. Mod Pathol. 2015 December 1;28(12):1594–602.
21. Huang W, Liu J, Xu K, Chen H, Bian C. PD-1/PD-L1 inhibitors for advanced or metastatic cervical cancer: From bench to bed. Front Oncol. 2022 October 14;12:849352.
22. Shao N. Research progress on human papillomavirus-negative cervical cancer: A review. Medicine. 2024 Oct 11;103(41):e39957.
23. Xie N, Shen G, Gao W, Huang Z, Huang C, Fu L. Neoantigens: promising targets for cancer therapy. Sig Transduct Target Ther. 2023 Jan 6;8(1):1–38.
24. Cervical cancer [Internet]. [cited 2023 September 11]. Available from: https://www.who.int/news-room/fact-sheets/detail/cervical-cancer
25. Lee JE, Chung Y, Rhee S, Kim TH. Untold story of human cervical cancers: HPV-negative cervical cancer. BMB Rep. 2022 September 30;55(9):429–38.
26. Xing B, Guo J, Sheng Y, Wu G, Zhao Y. Human Papillomavirus-Negative Cervical Cancer: A Comprehensive Review. Front Oncol. 2021 February 17;10:606335.