



KOILOCYTOSIS: DIFFERENTIATING TRUE FROM PSEUDO USING PERIODIC ACID-SCHIFF STAIN.

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ABSTRACT

Objectives: Distinguishing koilocytes from their mimickers using Periodic acid-Schiff Stain.

MATERIALS AND METHODS: This was a prospective analytical study. Data for the present study was obtained from the cervical biopsy specimens, received in the Department of Pathology, Mysore Medical College and Research Institute. Specimen received were fixed in 10% formalin and paraffin sections were prepared and stained with H&E for routine examination. The cases which showed koilocytic changes were stained with PAS Stain. Perinuclear vacuolation, nuclear changes and cytoplasmic characters were studied in both H&E and PAS-stained slides and the findings were recorded.

RESULTS: A total of 432 cases of koilocytosis on H & E stain were included in the study. The age of the patients ranged from 29- 63 years with maximum number of cases between 3rd and 4th decade. Least number of cases were observed before 30 years of age. Additional sections were obtained for these 432 cases and were stained with PAS stain. Only 54 cases showed PAS positivity and the remaining were PAS negative.

CONCLUSION: A diagnosis of koilocytes increases the need for frequent screening. But, overdiagnosis of HPV infection in cervical biopsies results in unnecessary diagnostic tests and surgical procedures, increased health care costs and mental trauma to the patient. Hence, HPV infection should be observed for a period of several months before definitive ablative treatment is undertaken.

Keywords: Koilocytic change, HPV infection, Periodic acid Schiff stain (PAS)

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INTRODUCTION:

Cervical cancer is the second most common cancer among women and one of the leading causes of cancer death among women worldwide. According to the World Health Organization (WHO) in 2018, an estimated 5,70,000 women were diagnosed with cervical cancer worldwide and about 3,11,000 women

died from the disease. Major risk factor is the Human papilloma virus (HPV) infection which is sexually transmitted and affects both the genders.¹ More than 200 types of HPV have been identified and are grouped into high risk and low risk based on their association with cervical cancer. High risk strains, HPV 16 and



HPV 18, are responsible for most of the HPV-related cancers.^{2,3}

The incidence is decreasing in different countries due to the introduction of screening methods and incorporation of vaccination in the immunization schedule. Community awareness, HPV vaccination, and effective screening methods have reduced the morbidity and mortality. Artificial Intelligence is an upcoming tool for early cancer screening. If detected early, carcinoma cervix can be treated effectively. Thus, reducing the morbidity and mortality. With comprehensive approach, cervical cancer can be eliminated from the world.

What are koilocytes and their look alike?

Koilocytes (Greek: koilos meaning hollow) are squamous cells with dysplastic nucleus and perinuclear halo.⁴Koilocytes are pathognomonic of low grade squamous intraepithelial lesion (LSIL).^{5,6}Koilocytes indicate HPV infection. HPV infects the immature basal cells and then replicates in the mature squamous cells, which later causes cleared out cytoplasm and nuclear changes.³Koilocytes cannot be identified on gross specimens. They can only be detected microscopically. Cervical cytology (Pap smears) and tissue biopsy show koilocytosis.

With Papanicolaou stain, the condensed cytoplasm can be pink, blue, or both. The perinuclear cavitation is transparent. The nucleus is enlarged, hyperchromatic with an irregular border .With the hematoxylin and eosin stain of fixed tissue, pink cytoplasm condenses around perinuclear cavitation, and nucleus exhibits hyperchromasia and membrane irregularity. Maturing squamous cells in the superficial or intermediate layers exhibit the koilocytic changes.⁷ Basilar squamous cells may be infected, but do not show the characteristics of koilocytes.⁸

Reactive squamous changes, post menopausal epithelial changes, Trichomonas and other infection and artefacts during slide

preparation are the major mimickers of koilocytes.⁹ These changes should be differentiated from actual koilocytes of HPV infection to prevent radical treatments.

This ambiguity can be overcome by performing Periodic Acid Schiff (PAS) stain on tissue specimen. This special stain provides rapid results, cost effective and suitable for laboratories with limited resources. Eventhough molecular tests are available for detecting HPV infection, koilocytic change remains fundamental to pathologists.

Thus, our study aims at distinguishing koilocytes from their mimickers and preventing unnecessary wastage of time and resources. Also prevent the emotional burden to the patient.

MATERIALS AND METHODS

This present study was carried out in the department of pathology on cervical biopsy specimens done for various reasons. We received a total of 1215 cervical biopsy samples from 1st January 2023 to 31st December 2023(1 year). Out of which 432(35.56%) cases showed koilocytic change with hematoxylin and eosin stain. We selected these 432 cases and stained with Periodic Acid Schiff (PAS) stain. Perinuclear vacuolation, nuclear changes and cytoplasmic characters were studied in both H&E and PAS-stained slides and the findings were recorded.

Inclusion criteria: All cases with koilocytic change

Exclusion criteria: Cases of HSIL (high grade squamous intraepithelial neoplasia) and carcinoma cervix

RESULTS

A total of 432 cases of koilocytosis on H & E stain were included in the study. The age of the patients ranged from 29- 63 years with maximum number of cases between 3rd and 4th decade. Least number of cases were observed before 30 years of age. (Table 1)

Table no.: 01- Age wise distribution of cases with koilocytosis

AGE	NO. OF CASES	Percentage
< 30	12	2.77
31-40	191	44.21
41-50	173	40.04
51-60	35	8.11
>60	21	4.86
Total	432	100

The most common clinical presentation being leucorrhoea followed by irregular bleeding (Table 2).

Table no.: 02- Clinical presentation of cases

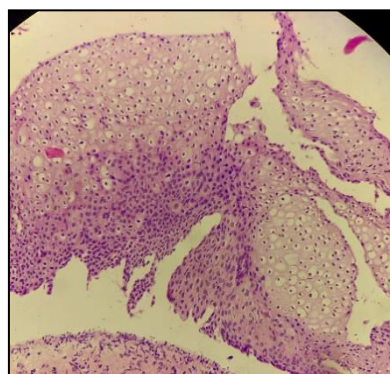
Age	Leucorrhoea	Irregular bleeding	Abdominal pain	Post coital bleeding	Mass per vagina	TOTAL
< 30	06	03	02	01	--	12
31-40	69	58	46	18	--	191
41-50	58	56	48	08	03	173
51-60	12	07	08	--	08	35
>60	--	05	04	--	12	21
Total	145	129	108	27	23	432

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Additional sections were obtained for these 432 cases and were stained with PAS stain. 378 cases showed PAS positivity and the remaining 54 were PAS negative as shown in table 3.

Table no.: 03- PAS positive and PAS negative cases

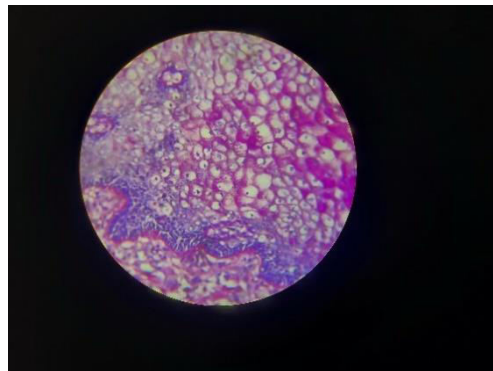
AGE	Koilocytosis	PAS -ve		PAS +ve	
		Number	%	Number	%
< 30	12	01	8.33	11	91.66
31-40	191	21	10.99	170	89.01
41-50	173	27	15.6	146	84.39
51-60	35	04	11.42	31	88.57
>60	21	01	4.75	20	95.23
Total	432	54	12.5	378	87.5



Fg.no 1 Koilocytosis on H&E stain



Fg.no 2 PAS negative



Fg.no3 PAS positive

DISCUSSION:

Koilocytosis, in cervix, is considered as marker for HPV infection and it aids in further diagnostic decision-making. In our study, out of 432 cases, majority of the cases were between 3rd and 4th decade indicating the higher incidence of HPV infection among the young. But koilocytosis is often transient as the immune system clears off the infection. Hence majority of the cases regress spontaneously. Only few cases progress to carcinoma. CIN 1 lesions with koilocytosis show lack of progression to CIN 2 and 3 lesions. Hence koilocytes are negative predictor of progression.⁷

About 4.86% (21 out of 432) of cases were seen in menopausal women. Postmenopausal cervix may also exhibit cellular alterations like perinuclear halos, nuclear hyperchromasia, variation in nuclear size, and multinucleation.⁸ It is very difficult to determine whether it is age-related epithelial changes or human papillomavirus (HPV)-related.

Majority of the cases (145 out of 432, 33.56%) presented with white discharge per vagina (leucorrhea) indicating that Trichomonas and other infections can also show small, non-specific halos around nucleus. Reactive squamous epithelium can also have vacuolar appearance, which mimics koilocytes. Thus, raising a suspicion of cervical cancer. There can also be artifactual koilocytes during slide preparation.

This method of doing an additional PAS stain on koilocytes overcomes all these problems and directs towards prompt treatment. For all the 432 cases of koilocytosis, additional sections were obtained and stained with PAS stain. Out of which 378 cases were PAS positive and only 54 cases were PAS negative.

PAS stain is used to assess the presence of neutral mucosubstance in the endocervical glands. PAS stain gives magenta colour with strong reactivity for normal endocervix

suggestive of presence of neutral mucin. But for adenocarcinoma cervix, PAS gave mild reaction as focal magenta staining. It is suggestive of presence of minimal neutral mucosubstances. Thus mucin patterns serve as valuable, cost-effective tool for early diagnosis of disease process.¹⁰

Hence we used PAS technique to know the alteration in mucin pattern to differentiate true koilocytes from their look alike on H&E stain.

Out of 432 cases of koilocytosis on H&E staining, only 54 cases were PAS negative suggesting true koilocytosis. Majority of the cases were seen between 4th and 5th decade indicating the high incidence of cervical cancer in this age group.

This method will save the remaining 378 patients from undergoing unnecessary procedures, reduce the economic and emotional burden. Once the PAS stain is positive, other causes of koilocytosis can be assessed. Only the cases with true koilocytes will be proceeded for further evaluation for carcinoma cervix. Thus reducing the case burden on healthcare team.

Another drawback is that the reproducibility of koilocytosis assessment is not optimal. There is lack of stringent criteria for diagnosis of koilocytes. Hence more objective criteria are necessary to avoid overdiagnosis, especially if the diagnosis rests on interpretation of koilocytotic atypia. The suboptimal interobserver reliability in the identification of koilocytes can also be minimized by PAS stain.

PREVENTION

Cervical cancer is curable cancer if detected early. In spite of which there has been significant morbidity and mortality. Vaccination offers reliable and long-term defence against HPV infection. Two vaccines are available in India, Gardasil and Cervarix. But there is lack of awareness and knowledge among people and also India lacks a national immunization program for carcinoma cervix eradication.^{11,12} Community awareness,

accessibility and affordability will definitely increase the vaccination coverage.

LIMITATIONS

There has not been any study of this kind of performing special stains on cases of koilocytosis. This limits us for any comparisons or references. Performing special stains are relatively easy and cost-effective procedures in centres with limited resources. It provides rapid results and directs towards correct treatment.

CONCLUSION

A diagnosis of koilocytes increases the need for frequent screening. But, overdiagnosis of HPV infection in cervical biopsies results in unnecessary diagnostic tests and surgical procedures, increased health care costs and mental trauma to the patient. Hence, HPV infection should be observed for a period of several months before definitive ablative treatment is undertaken. Prompt and timely treatment results in best possible outcome.

Conflict Of Interest: The authors declare no conflict of interest.

Funding: No funding was received to conduct this study.

Ethical Approval: The Institutional ethical committee of Mysore medical college and research institute and associated hospital mysore (EC REG: ECR/134/Inst/KA/2013/RR-19).

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References :

1. Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003 Jan;16(1):1-17. doi: 10.1128/CMR.16.1.1-17.2003. PMID: 12525422; PMCID: PMC145302.
2. Bean SM, Chhieng DC. Anal-rectal cytology: a review. Diagn Cytopathol. 2010 Jul;38(7):538-46. [PubMed]

3. Jeronimo J, Massad LS, Schiffman M., National Institutes of Health/American Society for Colposcopy and Cervical Pathology (NIH/ASCCP) Research Group. Visual appearance of the uterine cervix: correlation with human papillomavirus detection and type. *Am J Obstet Gynecol.* 2007 Jul;197(1):47.e1-8. [PubMed]
4. Koss LG. The 57th birthday of koilocytes. *Cancer Cytopathol.* 2012 Dec 25;120(6):421. [PubMed]
5. Groves IJ, Coleman N. Pathogenesis of human papillomavirus-associated mucosal disease. *J Pathol.* 2015 Mar;235(4):527-38. [PubMed]
6. Krawczyk E, Suprynowicz FA, Liu X, Dai Y, Hartmann DP, Hanover J, Schlegel R. Koilocytosis: a cooperative interaction between the human papillomavirus E5 and E6 oncoproteins. *Am J Pathol.* 2008 Sep;173(3):682-8. [PMC free article] [PubMed]
7. Meisels A, Fortin R. Condylomatous lesions of the cervix and vagina. I. Cytologic patterns. *Acta Cytol.* 1976 Nov-Dec;20(6):505-9. [PubMed]
8. Pinto AP, Crum CP, Hirsch MS. MOLECULAR MARKERS OF EARLY CERVICAL NEOPLASIA. *DiagnHistopathol (Oxf).* 2010 Oct 01;16(10):445-454. [PMC free article] [PubMed]
9. Krause KA, Neelon D, Butler SL. Koilocytosis. [Updated 2023 Aug 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK532958/>
10. Periodic Acid Schiff (PAS) Staining: A Useful Technique for Demonstration of Carbohydrates. (2020). *Medico Legal Update*, 20(4), 1370-1374?. <https://doi.org/10.37506/mlu.v20i4.2020>
11. A systematic review of interventions to improve HPV vaccination coverage. Mavundza EJ, Iwu-Jaja CJ, Wiyeh AB, Gausi B, Abdullahi LH, Halle-Ekane G, Wiysonge CS. *Vaccines (Basel)* 2021;9:687. [PMC free article] [PubMed] [Google Scholar]
12. Kaarthigeyan K. Cervical cancer in India and HPV vaccination. *Indian J Med Paediatr Oncol.* 2012 Jan;33(1):7-12. doi: 10.4103/0971-5851.96961. PMID: 22754202; PMCID: PMC3385284.