A Study of Nuclear Markers of Chromosomal Instability In Conventional Cervical Pap Smear

[1*]Dr. Shashikala C, [2]Dr. S. Brindha, [3]Dr. Dipti Kolhar
Assistant Professor,
Department of Pathology, Annapoorana Medical College And Hospitals,Salem

*Corresponding Author:
Dr. Shashikala C
Assistant Professor, Department of Pathology, Annapoorana Medical College And Hospitals, Salem

Abstract

Introduction

Cervical cancer remains second most common cancer among the women worldwide There are many ongoing researches to find newer screening tools in early diagnosis of CIN and carcinoma. Carcinogenesis affects cells by altering genetic material and thus causing chromosomal instability. Chromosomes or chromosome segments that fail to be incorporated into the nuclei during cell division configure as micronucleus [MN], nuclear budding [NB], and chromatin bridges [CB]. They can function as sensitive indicator of neoplastic lesions. This study aims to assess the morphologic markers of Chromosomal instability, a simple procedure in cytology smear that can be used to stratify the preinvasive and invasive tumors of uterine cervix. Also, to assess the scoring of morphologic markers of chromosomal instability in increasing grade of CIN and cervical carcinoma and its usage as screening tool.

Materials and Methods

Exfoliated cervical cells samples received at the department of pathology, Annapoorana medical College, Salem. during the study period July 2020 to June 2021 was studied. The cases were classified according to Bethesda 2014 guidelines for reporting cervical cytology. The total number of MN, NB and binucleated squamous cells with atypical nuclear features were counted per 1000 squamous epithelial cells. CB was counted per smear. Then the score of morphologic markers of chromosomal instability including MN, NB, CB and BN were compared with the spectrum of cervical lesions classified under 2014 Bethesda system.

Results

Among 292 cases, 237 [89.1%] were neoplastic and 29 [10.9%] cases were non-neoplastic. Within the neoplastic cases 21 [72.5%] cases were CIN and 8 [27.5%] cases were IC. The mean MN score showed increasing trend towards malignancy and was significantly more in neoplastic [4.86] when compared to non-neoplastic lesions [1.52]. The mean value of MN score was significantly more in IC [6.0] than CIN lesions [4.2]. NB was not seen in non-neoplastic conditions. Within the neoplastic conditions the mean NB was in increasing order from LSIL [1.0] to HSIL [1.67] to IC .One CB was noted in each case of HSIL and IC, indicating that they are evident in high grade neoplastic lesions. The mean BN was highest in invasive carcinoma

Conclusion

Thus the evaluation of MN, NB, CB and BN on exfoliated cervical cells can serve as a cost-effective additional criterion for establishing the cervical cancer in patients with risk

Keywords: Cervical cancer, Chromosomal instability; Micronucleus; Nuclear budding; Binucleate cell

Introduction

Cervical cancer remains second most common cancer among the women worldwide. It accounts for 500,000 new cases and 270,000 deaths across the globe ever year[1] In India alone there are estimated 132,000 new cases and 74000 deaths each year and accounts for 20 per 100,000 women in India.[2] Eighty percent of cervical cancer cases occurring in developing countries like India are because of lack of population-based screening. Hence newer protocol and techniques for cytologic screening of cervical smears are to be investigated which can render towards a goal of 100% early detection and appropriate treatment. Chromosomal instability [CI] manifested by increased aneuploidy and structural chromosomal aberrations is believed to play a critical role in the intermediate to late stages of the development of cervical malignancies. Chromosomes or chromosome segments that fail to be incorporated into the nuclei during cell division configure as micronuclei [MNi], nuclear budding [NB], and chromatin bridges [CB]. Thus, MNi, NB and CB represent a measure of both chromosome breakage and chromosome loss, and can function as sensitive indicator of genetic damage. Their frequency appear to increase in carcinogen exposed tissues long before any clinical symptoms are evident.[3]

Many studies have shown that mitotically unstable chromosomes identified by fluorescent in-situ hybridisation technique in cell cultures of malignant tumours showed MNi, NB and CB in interphase nuclei than from stable chromosome and can be regarded as an indicator of CI.[4,]A simple procedure of counting micronuclei in a cytology smear can be used to stratify the preinvasive disease of the uterine cervix. A recent study proved that micronucleus [MN] scoring can be performed satisfactorily in routine Pap smear.[2] The assessment of morphologic markers of CI with routine cytological analysis of conventional Pap smears is a cost effective and non-invasive method which can be a useful biomarker for the detection of human cancer at risk in organs from where exfoliated cells can be obtained.[5]

This study aims to assess the morphologic markers of CI, a simple procedure in cytology smear that can be used to stratify the preinvasive disease of uterine cervix also to assess the utility of count of morphologic markers of chromosomal instability as a screening tool in diagnosis of CIN and cervical cancer.

Materials And Methodology

Exfoliated cervical cells samples received at the Department of Pathology, Annapoorana Medical College Hospital, Salem during the study period of July 2020 to June 2021 was studied. Clinical details were obtained from the patients and out-patient record¸ after obtaining written informed consent after getting ethical approval. Thin smears were prepared from each sample on a glass slide, which were fixed in 95% alcohol for 10-15 minutes and stained with conventional Papanicolaou stain. Interpretation of the smear was done according to the 2014 Bethesda system.[6]

Under oil immersion, 1000 squamous epithelial cells were examined and the total number of MN, NB and binucleated [BN] squamous cells with atypical nuclear features were counted. CB was counted per smear. Clumps of cells with obscured nuclear or cytoplasmic boundaries and overlapping of cells were avoided and cells lying singly were preferred for counting the markers of CI. Degenerated cells, apoptotic cell and cytoplasmic fragments were exempted from counting and scoring. The zig-zag method was followed for screening the slides.[5]

The collected data was entered and analyzed using Epi-info software and descriptive statistics like mean, median, standard deviation. Percentage and Chi-square test to know the association. The p value of <0.05 was considered statistically significant.

Results

In the present study 305 cases of exfoliated cervical smears were of which 13 cases were excluded because of poor cellularity and 292 cases were analyzed. Of the total 292 cases of cervical Pap smear examined 276 [94.5%] cases were premenopausal and 16 [5.5%] were post-menopausal patients. In the present study, of the 292 cervical Pap smear 156 [53.4%] patients presented with white discharge per vagina followed by pain abdomen, bleeding per vagina, and mass per vagina.

Of the 292 cases analyzed microscopically as per Bethesda system 2014, 55 [18.8%] cases had normal cervical morphology, 67 [22.9%] cases were of

infections, 112 [38.4%] cases were inflammatory, 3[1%] cases were atrophy, 19 [6.5%] cases were ASCUS, 6 [2.1%] cases were ASC-H, 1[0.3%] case was Atypical endocervical cells, 14 [4.8%] cases were LSIL, 7 [2.4%] cases were HSIL, and 8 [2.7%] cases were of invasive carcinoma [IC]

A total of 67 cases of infection were diagnosed, of which 22 were trichomonas vaginalis infection, 26 were candida species infection, 15 cases were bacterial vaginosis, 3 cases were both trichomonas vaginalis and candida species infection and 1 case was HSV. Trichomonas vaginalis infection were identified by following features Pear shaped, oval or round cyanophilic organism Nucleus is pale vesicular and eccentrically located.

The median age among the cases of normal, infections, inflammatory, atrophy, ASCUS, ASC-H, AEC, LSIL, HSIL and invasive carcinoma were 34, 32, 31.5, 49, 38, 41.5, 56, 41 and 45 respectively. The median parity was 1 in normal, infection and inflammatory patients, 2 in cases of ASCUS, ASC-H and LSIL patients and 3 in cases of atrophic, AEC, HSIL and invasive carcinoma patients.

Normal, infections, inflammatory, and atrophic cases were categorized under non-neoplastic lesions and the neoplastic lesions comprised of LSIL, HSIL and IC for comparison study. The lesions of ASCUS, ASC-H and AEC were grouped separately. Number of non-neoplastic conditions and neoplastic conditions were 237 [89.1%] and 29 [10.9%] respectively. Of the 29 neoplastic cases, 21 [72.5%] cases were CIN lesions [LSIL and HSIL] and 8 [27.5%] cases were of IC.

The micronuclei [MN] were seen in various lesions. The MN score/1000 cells in various categories showed a MN range of 1, 1 to 3, 1 to 3, 1, 2 to 5, 2 to 4, 2 to 6, 3 to 6 and 2 to 8 in normal, inflammatory, infection, atrophy, ASCUS, ASC-H, LSIL, HSIL and IC respectively. This revealed that the range of MN score was significantly higher in LSIL, HSIL and IC categories than the normal, inflammatory, infectious and atrophic categories. There was an intermediate

increase in range on MN score in ASCUS and ASC-H categories. It is evident by the above data that the mean and median value of MN score is more in CIN and IC when compared to non-neoplastic conditions. Within the neoplastic category the mean and median value of MN score was more in IC than CIN lesions.

No nuclear budding [NB] was seen in normal, inflammatory, infection, atrophy, AEC and ASC-H category when 1000 cells were examined. NB was seen in neoplastic conditions and was seen in 26.3% [5/19 cases] of ASCUS, 50% [7/14 cases] of LSIL, 85.7% [6/7 cases] of HSIL and 87.5% [7/8 cases] of IC categories. A single NB was seen in 5 cases of ASCUS and 7 cases of LSIL with the mean NB score of 1in both. In HSIL and IC the range of NB were 1 to 2 and 1 to 3 respectively with mean $\pm$ SD of NB being 1.67 $\pm$ 0.52 and 2.43 $\pm$ 0.78 respectively. This data revealed that NB was found in neoplastic conditions [Fig 36, 37] apart from ASCUS and not in non-neoplastic conditions. Within the neoplastic conditions the range of NB and mean $\pm$ SD of NB was more in HSIL and IC categories than LSIL.

Chromatin bridges [CB] was evident only in HSIL and IC [Fig 39] and in both only a single CB was found signifying that chromatin bridges are seen only in high grade CIN and IC.

Binucleated [BN] cells with atypical nuclear features were not seen in normal, inflammatory, infection, atrophic categories. The atypical BN were seen neoplastic conditions [Fig 40, 41] and the mean $\pm$ SD of BN in ASCUS, ASC-H, LSIL, HSIL and IC were 3.84 $\pm$ 1.77, 3.17 $\pm$ 0.98, 4.78 $\pm$ 1.05, 3.43 $\pm$ 1.27 and 6.12 $\pm$ 0.83 respectively. The above data indicates that the mean $\pm$ SD of BN was high in IC than the CIN categories.

Comparison of mean MN score between non-neoplastic and neoplastic conditions showed a mean value of 1.52 and 4.86 respectively indicating that the mean MN score is more in neoplastic categories. The p value was statistically significant [<0.0001] between non-neoplastic and neoplastic conditions.

Table 1: Comparison of MN score between neoplastic and non-neoplastic lesions

Lesions	Number of cases	Cases with MN	Mean MN	SD	p value
Non-neoplastic	237	80	1.52	0.63	
Neoplastic	29	29	4.86	1.50	<0.0001

The NB was noted only in neoplastic conditions and showed a mean score value of 1.7 indicating that the mean NB score is more in neoplastic categories. Statistical significance could not be evaluated as there was no NB in non-neoplastic lesions.

Table 2: Comparison of mean NB score between neoplastic and non-neoplastic lesions

Lesions	Number of cases	Cases with NB	Mean NB	SD
Non-neoplastic	237	00	-	-
Neoplastic	29	20	1.7	0.80

The CB was not found in non-neoplastic cases. As the CB was noted only among neoplastic conditions, the comparison of mean CB score between non- neoplastic and neoplastic could not be evaluated.

Table 3: Comparison of mean CB score between neoplastic and non-neoplastic lesions

Lesions	Number of cases	Cases with CB	Mean CB	SD
Non-neoplastic	237	0	0	0
Neoplastic	29	2	1	-

The BN was not found in non-neoplastic cases. As the BN was noted only among neoplastic conditions, the comparison of mean CB score between non- neoplastic and neoplastic could not be evaluated.

Table 4: Comparison of mean BN score between neoplastic and non-neoplastic cases

Lesions	Number of cases	Cases with BN	Mean BN
Non-neoplastic	237	00	-
Neoplastic	29	29	4.8

The mean MN score in CIN cases was 4.2 and in IC cases it was 6.0 suggesting a slight increase in a mean MN score in the later category with a statistically significant p value [0.002].

The mean NB score in CIN cases was 1.3 and in IC cases it was 2.43 suggesting an increase in the mean NB score in the IC with a statistically significant p value [<0.001]. CB score was equal [1] among CIN and IC. The mean of CB could not be evaluated as only one case of each of the CIN and IC categories showed CB. The mean BN score of IC lesions were high compared to CIN lesions and it was statistically significant [p<0.001].

Page 1012

The mean MN score was 4.21 in LSIL category and 4.86 in HSIL category suggesting a slight increase in mean MN score in HSIL category. However, the p-value was not statistically significant [0.1976]. The mean NB score of LSIL was 1.0 compared to 1.7 in HSIL category suggesting an increase in the later category. We could not determine the statistical

significance as there was no SD in the mean NB of LSIL cases. No CB was seen in LSIL category and 1 CB was found in HSIL category. In contrast to the other markers the mean BN score of LSIL category [4.78] was high compared to HSIL [3.43] category. However, the p value was not statistically significant [0.178]

Figure 1: Micronuclei and Chromatin bridge
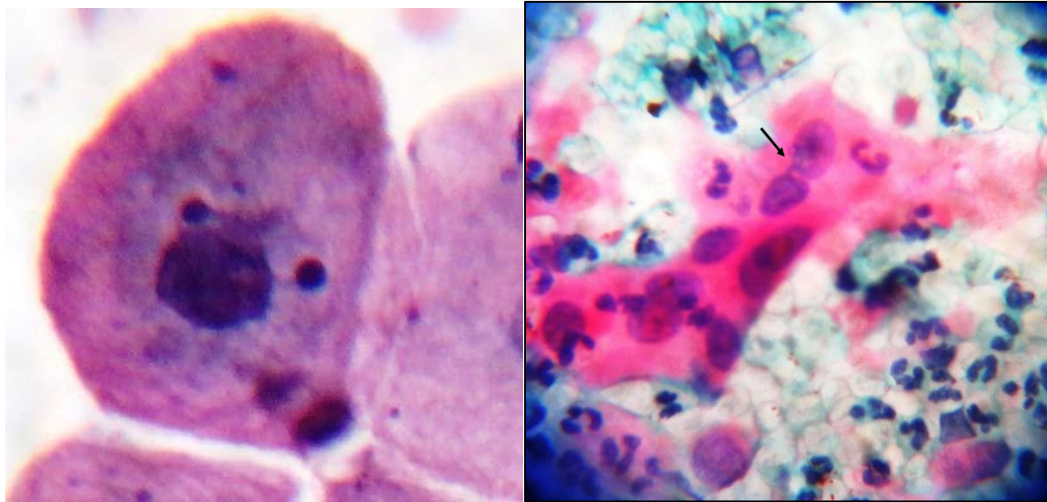
Discussion

Our study evaluated the frequency of the morphological markers of the chromosomal instability which includes micronuclei, nuclear budding, chromatin bridges and atypical binucleated cells in 292 cases of cervix smears of patients with various lesions. These findings were correlated with other studies. In present study, of the 292 patients most of them were in premenopausal age group [99.2%]. However, in the study done by Deeksha Pandey et al[7] 65% of patients were in premenopausal status. In our study all the cases [100%] of women in IC group attained menopause and 0.8% attained menopause in non-neoplastic group. Similarly study by Deeksha Pandey et al[7] showed a high percentage [71.9%] of postmenopausal women in malignant group.

The present study and the study conducted by Gayathri et al,[5] Samantha et al[8] and Yong-Hua shi et al[9] showed that the non-neoplastic conditions like normal, infection and inflammatory conditions occurred in younger age group and invasive carcinoma occurred in elderly individuals. The CIN lesions occurred in middle age group patients.

The mean MN $\pm$ SD of various categories in the present study revealed that the mean MN $\pm$ SD was significantly higher in LSIL, HSIL and IC categories than the normal, inflammatory, infectious and atrophic categories. There was an intermediate increase in range on MN score in ASCUS and ASC-H categories. Our results were similar to study done by Gayathri et al5, Samantha et al[8], Deeksha Pandey et al[7] and Ambroise et al[10].

In the study by A.Hemalatha et al[11] done to asses MN score utility in breast aspirates, the mean MN was significantly high in infiltrative ductal carcinoma compared to fibroadenoma. Similarly in the present study mean MN score was more in CIN and IC cases than non-neoplastic cases.

Comparison of mean MN between neoplastic and non-neoplastic conditions revealed a significant increase of mean MN in neoplastic compared to non-neoplastic conditions. Various other studies done by Ambroise et al[10] ,Samantha et al[8]& Bueno et al[12] also had the similar findings. Study of MN index in premalignant and malignant lesions of oral cavity by Sivasankari.P et al[13] showed that the mean MN was high in the malignant cases compared with

Page 1013

premalignant cases. Similar results were seen in the present study in which mean MN in IC cases was high compared to CIN [LSIL and HSIL] cases.

Study of NB and CB as biomarker of CI has not been done in cervical smears by any of the authors till now. We are the first to study NB and CB scoring in cervical smears and establishing the utility as morphological markers of CI. However, few authors in their study of breast lesions and ascitic fluid have scored NB and CB and established them as markers of CI.

Tyagi R et al[14] analyzed the significance of morphological markers of CI [MN, NB, CB and multipolar mitosis] in malignant and benign ascitic fluid smears. They concluded that there is strong correlation between markers of CI and cytological diagnosis of malignancy and in future, the knowledge of these markers can be applied to diagnose malignancy in suspected cases of effusion in difficult situation.

In present study NB was seen in only neoplastic conditions and a single CB was found each in HSIL and Invasive carcinoma suggesting that their presence indicates a strong cytogenetic damage in cervical epithelium by HPV. Therefore, NB and CB can be used as biomarker of CI in predicting the prognosis of various cancers. Though the MN were seen in normal and non-neoplastic conditions, it was their increasing frequency of occurrence which was more striking in CIN and IC, and hence MN scoring can be used to predict the outcome of non-neoplastic and neoplastic conditions.

In present study there were no BN in normal, infections, inflammatory and atrophic smears and the mean BN was highest in IC when compared to CIN. In contrast, the study by Deeksha Pandey et al[7] and Ambroise et al[10] showed the presence of BN in normal, infection, inflammatory and atrophic smears. This could be explained by the possibility that we counted only the BN cells with atypical nuclear features.

Conclusion

The present study has shown a strong correlation between markers of chromosomal instability and cytological diagnosis of CIN and IC. Since the NB and CB were found only in CIN and IC they are more potential marker of CI than MN. To conclude,

scoring of MN, NB, CB and BN on epithelial cells could be used as a biomarker in cancer screening. This is a simple, reliable, reproducible and objective test and can serve as an effective biomarker in conjunction with the conventional cervical Pap screening as per Bethesda 2014 for early diagnosis of CIN and cervical cancer.

References

1. Torre L.A, Bray. F, Siegel. R.L, Ferlay. J, LortetTieulent. J, Jemal. A. Global Cancer Statistics, 2012. CA Cancer J Clin. 2015;65:87–108.

2. Gandhi G, Kaur B. Elevated frequency of Micronuclei in uterine smears of cervix cancer patients. Caryologia. 2003;56:217-22.

3. Lízia Maria Franco dos Reis Campos, Francisca da Luz Dias, Lusânia Maria Greggi antunes, Eddie Fernando Candido Murta. Prevalence of micronuclei in exfoliated uterine cervical cells from patients with risk factors for cervical cancer. Sao Paulo Med J 2008;126(6):323-8.

4. French M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, et al.Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 2011;26(1):125-32.

5. Gayathri BN, Kalyani R, Hemalatha A, Vasavi B. Significance of micronucleus in cervical intraepithelial lesions and carcinoma. Journal of Cytology 2012; 29(4):236-40.

6. Solomon D, Nayar R, Wilbur DC. The Bethesda system for reporting cervical cytology: Definitions, criteria, and explanatory notes. 3rd ed. New York: Springer; 2015.

7. Deeksha Pandey, Sahitya Putteddy, Satish Rao. Micronucleus assay as a triage tool for borderline cases of cervical dysplasia. Sri Lanka Journal of Obstetrics and Gynaecology 2011;33:104-11.

8. Samanta S, Dey P, Nijhawan R. Micronucleus in cervical intraepithelial lesions and carcinoma. Acta Cytologica 2011;55:42-7

9. Yong-Hua Shi, Bo-Wei Wang, Talaf Tuokan, Qiao-Zhi Li, Ya-Jing Zhang.Association between micronucleus frequency and cervical intraepithelial neoplasia grade in Thinprep cytological test and its significance. International Journal of Clinical Experiment in Pathology 2015;8(7):8426-32.

10. Marie Moses Ambroise, Kanchana Balasundaram, Manjiri Phansalkar. Predictive Value of Micronucleus Count in Cervical Intraepithelial Neoplasia and Carcinoma. Turkish Journal of Pathology 2013;29:171-8.

11. Hemalatha TN Suresh, Harendra ML Umar. Micronuclei in breast aspirates.Is scoring them helpful? Journal of Cancer Research and Therapeutics 2014 Apr-Jun;10(2).309-11.

12. Bueno CT, Cláudia Dornelles da Silva M, Barcellos RB. Association between cervical lesion grade and micronucleus frequency in the Papanicolaou test. Genetics and Molecular Biology 2014;37(3):496-9.

13. Sivasankari PN, Kaur S, Reddy KS, Vivekanandam S, Ramachandra Rao K. Micronucleus Index: An Early Diagnosis In Oral Carcinoma. Journal of Anatomy Society 2008;57(1):8-13.Tyagi R, Dey P, Uppal R, Rajwanshi A. Analysis of morphological markers of chromosomal instability in ascitic fluid. Diagnostic Cytopathology 2015 Oct; 43(10):855-8