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Original Research Article

A Study of Morphological Markers of Chromosomal Instability in a Spectrum of Cervical Epithelial Lesions and its Correlation with 2014 Bethesda System

Shivakumar S.[1], Vedavathi B.L.[2]

[1]Professor and Head [2]Post Graduate Student, Department of Pathology, Mandya Institute of Medical Sciences, Mandya, Karnataka 571401, India.

Corresponding Author:
Vedavathi B.L., Post Graduate Student, Department of Pathology, Mandya Institute of Medical Sciences, Mandya, Karnataka 571401, India.

E-mail: vedavathi.lakshman@gmail.com

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Abstract

Introduction: Chromosomes or chromosomal segments that fail to get incorporated into nuclei during cell division configure as micronuclei (MN), nuclear budding (NB) and chromatin bridges (CB). The frequency of these markers are increased in carcinogen-exposed tissues long before any clinical symptoms are evident. *Objectives of the Study:* To study and compare the scoring of morphological markers of chromosomal instability in a spectrum of non-neoplastic, intraepithelial intraepithelial neoplasm and invasive cancer of the cervix. *Methodology:* Retrospective study conducted in the department of pathology, MIMS, Mandya from January 2016 to December 2017. All conventional pap smears with epithelial cell abnormalities and equal number of age matched pap smears without epithelial cell abnormalities received during study period were interpreted according to 2014 Bethesda system and examined for MN, NB and CB. *Results:* Out of 150 conventional pap smears studied 53 smears showed neoplastic changes and 75 were non-neoplastic. Carcinoma in situ (LSIL and HSIL) seen in 42 smears and invasive carcinoma in 11 smears. The mean MN score and NB score showed increasing trend towards malignancy with significant p value <0.05. The MN score and NB score is significantly high in invasive carcinoma compared to carcinoma in situ. CB was observed in one case each of LSIL, HSIL and IC. *Conclusion:* This is a simple, reliable, reproducible and cost-effective test and can serve as an effective biomarker in conjunction with the conventional cervical Pap screening for early diagnosis of CIN and cervical cancer.

Keywords: Morphological Markers of Chromosomal Instability; HSIL; LSIL; Micronuclei; Nuclear Budding; Nuclear Bridging.

Introduction

Carcinogens affect cells by altering genetic material and thus causing instability. Chromosomal instability 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 chromosomal segments that fail to incorporated into nuclei during cell division configure as micronuclei (MN), nuclear budding (NB) and chromatin bridges (CB). Thus MN, NB and CB represent a measure of both chromosome breakage and chromosome loss, and can function as a sensitive indicator of genetic damage [1].

In a gradient of cervical neoplastic lesions from low to high-grade is characterized by increasing nuclear atypia and the failure of cellular differentiation. These phenotypic changes are presumed to be accompanied by increased genetic instability that can be documented using the micronuclei (MN) assay in exfoliated cervical cells[2].

The MN mainly originate from acentric chromosome fragments, acentric chromatid fragments or whole chromosomes that fail to be included in the daughter nuclei at the completion of telophase during mitosis [3].

The CB originates during anaphase when the centromeres of dicentric chromosomes are pulled to opposite poles of the cell during mitosis [3].

NB has been observed in cultures grown under strong selective conditions, which induce gene amplification as well as under moderate folic acid deficiency [3].

Morphological features like micronuclei, nuclear bridging, nuclear budding and multipolar mitosis are well evaluated markers for chromosomal instability [4]. The frequency of these markers are increased in carcinogen-exposed tissues long before any clinical symptoms are evident [4].

The molecular methods to determine chromosomal instability are costly, require expertise and may not be available in many laboratories. These morphological markers can be use to assess chromosomal instability in intraepithelial neoplasm and invasive cervical carcinoma [2].

Objectives of the Study

To study the morphological markers of chromosomal instability in a spectrum of non-neoplastic lesions, intraepithelial neoplasm and invasive cancer of the cervix.

To compare the scoring of morphologic markers of chromosomal instability in increasing grades of cervical intraepithelial neoplasm and cervical carcinoma.

Materials and Methods

The present cross sectional and observational study conducted in the department of pathology, MIMS, Mandya including all the conventional pap smears with epithelial cell abnormalities, which were satisfactory for evaluation and equal number of pap smears without epithelial cell abnormalities received between January 2016 to December 2017 for a period of 2 years. Unsatisfactory smears were excluded. Clinical details are obtained from the medical records and request forms. The conventional pap smears received during study period were retrieved and interpreted according to 2014 Bethesda system [5]. Under oil immersion [x100], 1000 squamous epithelial cells were examined and the total number of MN, NB and CB present were noted. The score of micronuclei, nuclear budding and Chromatin Bridge compared with the spectrum of cervical lesions classified under 2014 Bethesda system.

Plan of Data Analysis

Data collected is entered in Excel sheet and analysed using Epi/SPSS software and the descriptive statistics using Chi-square test, Student's t-test.

Results

Data obtained is tabulated and analysed. Studied 75 conventional pap smears with epithelial cell abnormalities received during study period along with 75 conventional pap smears without epithelial cell abnormalities (randomly selected). Out of 150 pap smears studied 53 (35.2%) were neoplastic. The mean age of presentation of NILM, ASCUS, ASC-H, AEC, LSIL, HSIL and IC were 39, 46, 50, 60, 45, 38 and 56 respectively. The micronuclei were seen in neoplastic, non-neoplastic, ASCUS, ASC-H and AEC lesions. All study subjects with epithelial abnormalities showed MN, range varied between 1-6. Whereas only 3 (4%) of NILM study subjects showed MN, range varied between 0-2. IC showed higher range of 5-6 (Table 1).

The NB were seen in neoplastic, non-neoplastic, ASCUS, ASC-H and AEC lesions. Out of 75 study

subjects with epithelial abnormalities NB is seen in 51 (34%), of pap smears and range varied between 1-2. Only 1 (1.3%) of NILM study subject showed NB, range varied between 0-2. Whereas NB is seen in all smears with IC with a range of 0-2 (Table 2). The CB were seen only in neoplastic lesions. CB is seen in 9% of smears with IC, 7.6% of smears with HSIL, 3.4% of smears with LSIL. Range

Table 1: Distribution frequency of MN among study subjects

Lesions	No of cases	Total	Percentage (%)	Range
NILM	3	75	4.0	0-2
ASCUS	14	14	100	2-4
ASC-H	3	03	100	3-4
AEC	5	05	100	1- 2
LSIL	29	29	100	2-5
HSIL	13	13	100	3-5
IC	11	11	100	5-6
Total	78	150	52%	0-6

Table 2: Frequency distribution of NB among study subjects

Lesions	No of cases	Total	Percentage (%)	Range
NILM	1	75	1.3	0-1
ASCUS	7	14	50	0-1
ASC-H	2	03	66.6	1-2
AEC	2	05	40	1-2
LSIL	18	29	62.0	02
HSIL	10	13	76.9	0-1
IC	11	11	100	1-2
Total	51	150	34	0-2

Table 3: Frequency distribution of CB among study subjects

Lesions	No of cases	Total	Percentage (%)	Range
NILM	0	75	0	0
ASCUS	0	14	0	0
ASC-H	0	03	0	0
AEC	0	05	0	0
LSIL	1	29	3.4	0-1
HSIL	1	13	7.6	0-1
IC	1	11	9.0	0-1
Total	3	75	04	0-1

Comparison of morphological markers of chromosomal instability between non neoplastic and neoplastic categories.

Table 4: Comparison of mean MN score between non neoplastic and neoplastic categories.

Lesions	No of cases	Total	Percentage (%)	Mean MN score	P value
Non-neoplastic	04	75	5.3	0.2	< 0.05
Neoplastic	53	53	100	3.4	

Table 5: Comparison of mean NB score between non neoplastic and neoplastic categories.

Lesions	No of cases	Total	Percentage(%)	Mean NB score	P value
Non-neoplastic	01	75	1.3	0	<0.05
Neoplastic	39	53	73.5	0.8	

varied between 0-1 (Table 3). The neoplastic lesions comprised of LSIL, HSIL and IC. ASCUS, ASC-H and AEC were grouped separately. MN were seen in all neoplastic lesions with a mean score of 3.4. Whereas only 04 (5.3%) of non-neoplastic smears showed MN with a mean score of 0.2. The MN in neoplastic lesions is statistically significant with a p value of < 0.05 (Table 4). NB is seen in 39 (73.5%) of neoplastic lesions with a mean NB score of 0.8. Whereas 1 (1.3%) of non-neoplastic smear showed NB. The NB in neoplastic lesions is statistically significant with a p value of < 0.05 (Table 5). CB is seen in 01(1.8%) of neoplastic lesion with a mean NB score of 0.03. Whereas none of the non-neoplastic smears showed CB (Table 6). All study subjects with CIN and IC lesion showed MN. Higher mean MN score of 5.4 is seen in IC (Table 7). All study subjects with IC showed NB, whereas 28 (66.6%) showed NB. Higher mean NB score of 1.7 is seen in IC (Table 8). The CB is seen in 01 (11%) of IC and 02(4.7%) of CIN study subjects and mean CB score is 0.05 and 0.07 respectively (Table 9).

Discussion

In the present study morphological markers of chromosomal instability like micronuclei, nuclear budding, chromatin bridging were studied in 75 conventional pap smears and 75 smears negative for intraepithelial lesion or malignancy. Several studies have evaluated the frequency of association

Table 6: Comparison of mean CB score between non neoplastic and neoplastic categories.

Lesions	No of cases	Total	Percentage(%)	Mean CB score
Non-neoplastic	0	75	0	0
Neoplastic	01	53	1.8	0.03

Comparison of morphological markers of chromosomal instability between CIN (LSIL +HSIL) and IC.

Table 7: Comparison of mean MN score between CIN (LSIL +HSIL) and IC.

Lesions	No of cases	Total	Percentage (%)	Mean MN score
CIN	42	42	100	3.5
IC	11	11	100	5.4

Table 8: Comparison of mean NB score between CIN (LSIL +HSIL) and IC.

Lesions	No of cases	Total	Percentage (%)	Mean NB score
CIN	28	42	66.6	0.6
IC	11	11	100	1.7

Table 9: Comparison of mean CB score between CIN (LSIL +HSIL) and IC.

Lesions	No of cases	Total	Percentage (%)	Mean CB score
CIN	02	42	4.7	0.05
IC	01	11	9.0	0.07

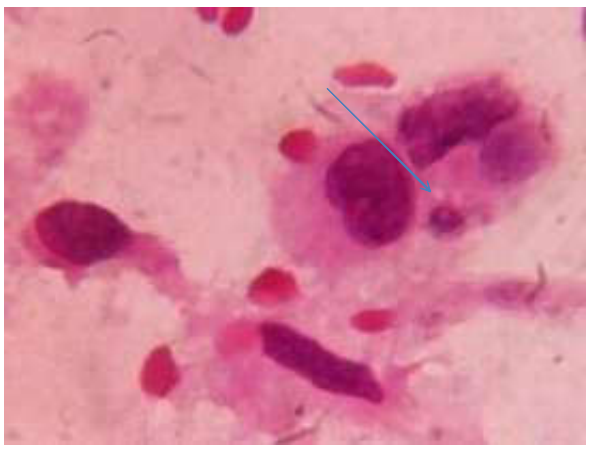

Fig. 1: Micronucleus (blue arrow) (H and E) 100X

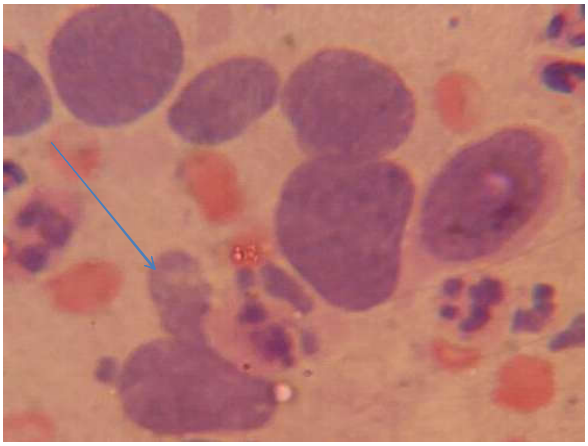

Fig. 2: Nuclear Budding (blue arrow) (H and E) 100X

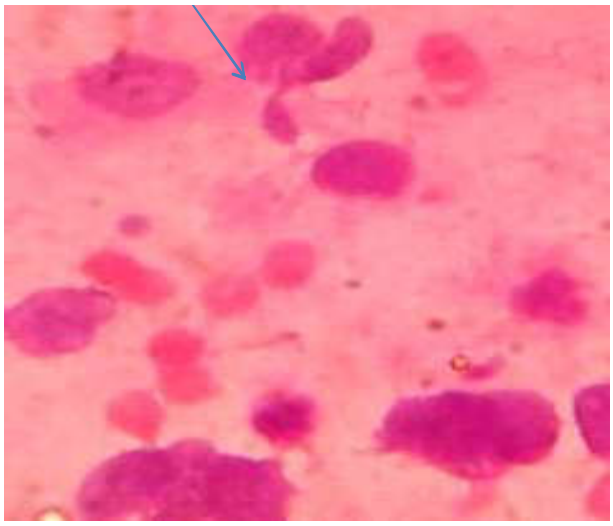

Fig. 3: Chromatin Bridging (blue arrow) (H and E) 100X

Table 10: Comparison of mean age of study subjects with other studies

Lesion	Gayathri et.al.[9]	Samantha et.al.[10]	Present study
NILM	45.2	41.0	39
ASCUS	44.0	-	46
AC-H	46.5	-	50
AEC	-	-	60
LSIL	43.5	41.1	45
HSIL	48.5	40.9	38
IC	56.5	52.7	56

Table 11: Comparison of mean MN between neoplastic and non neoplastic conditions with other studies

Study	Neoplastic	Non –neoplastic
Gayathri et.al.[9]	7.53	2.4
Samantha et.al.[10]	14.3	1.40
Ambroise et.al.[11]	10.9	2.1
Present study	3.4	0.8

Table 12: Comparison of mean MN between LSIL and HSIL non neoplastic conditions with other studies

Study	LSIL	HSIL
Gayathri et.al.[9]	4.1	8.0
Samantha et.al.[10]	4.7	19.7
Ambroise et.al.[11]	4.4	11.9
Present study	2.8	4.1

of micronuclei in a spectrum of cervical epithelial lesion. Nuclear budding and chromatin bridging were studied as markers of chromosomal instability in various neoplastic lesions except cervical cancer. However no study evaluated the frequency of association of nuclear budding and chromosomal instability in cervical smears.

In the present study, mean age of study subjects for LSIL, HSIL and IC is 45, 38 and 56 years respectively. Similar to study conducted by Gayathri et.al. of 43.5, 48.5 and 56.5 years for LSIL, HSIL and IC respectively (Table 10). Samantha et al. of 41.1, 40.9 and 52.7 years for LSIL, HSIL and IC respectively. The mean MN for neoplasic condition is more than the non neoplastic conditions which is comparable with study conducted by Gayathri et.al., Samantha et.al. and Ambroise et.al. (Table 11). The mean MN for HSIL is more than LSIL which is comparable with study conducted by Gayathri et.al., Samantha et.al. and Ambroise et al. (Table 12).

Conclusion

The molecular methods to determine chromosomal instability are costly, require expertise and may not be available in many laboratories. These morphological markers can be used to assess chromosomal instability in intraepithelial neoplasm and invasive cervical carcinoma.

Abbreviations:

NILM-Negative for intraepithelial lesion,
ASC-US-Atypical squamous cells of undetermined significance,
ASC-H -Atypical squamous cells cannot exclude HSIL,
LSIL-Low-grade squamous intraepithelial lesion,
HSIL -High-grade squamous intraepithelial lesion,
IC-Invasive carcinoma,
AEC- Atypical endocervical cells,
MN –Micronuclei,
NB-Nuclear budding,
CB-Chromatin bridges,
CIN-Cervical intraepithelial neoplasia.

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