Original Research Article

Micronucleus Test as a Prognostic Marker in Cervical Lesions on Pap smear

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Abstract: Cervical Cancer is the third most common type of cancer among women worldwide .Micronucleus is a small additional nucleus formed due to chromosomal loss of fragmentation .Their frequency appears to increase in carcinogen exposed tissue long before clinical symptoms are evident . The objective is to assess the utility of micronucleus count as a prognostic marker in cervical lesions on pap smear . In this retrospective study , we have compared the micronucleus score in the whole spectrum of cervical lesions comprising of seven groups like normal (30) , inflammatory (38), ASC-US (2), ASC-H(2), LSIL(20), HSIL(6), IC (2) over a period of one year , i.e from June 2015 – 2016. Histopathological correlation was done only for available cases. Two observers separately counted the number of micro nucleated cells per 500 epithelial cells in oil immersion magnification and where expressed as MN score per 500 cells . There was a stepwise gradual increase in micronucleus count from inflammatory to ASC-US to LSIL to HSIL and IC group. The mean MN scores ±SD in normal (2.57±1.38) ,inflammatory (8.21±1.26), ASC-US (10±2.83), ASC-H(13±2.83), LSIL(15.8±1.44), HSIL(20.33±1.63) and IC (23±2.83) cases of cervical lesions. The result was statically significant with p value <0.0001. MN counting can be helpful prognostic marker .This is easy , reliable ,simple, objective test which can be performed on routinely stained pap smear.

Keywords: Micronucleus, Cervical smears

INTRODUCTION

Cervical cancer is the most common cancer among women in developing countries and the second most common cancer among women globally. It is true that cervical carcinoma owes the most gratifying success story in the history of oncology till date. Credit goes to its long preinvasive phase, easy and applicable screening methods ,and effective treatment of preinvasive lesions. Pap smear are the most common and inexpensive method of screening for cervical cancer[1,2]. The estimated worldwide incidence of cervical cancer is approximately 500,000 new cases per year contributing to 2,70,000 deaths across the globe every year, out of these, the burden of 2,30,000 [85%] deaths occurs in developing countries. In India alone there are estimated 1,32,000 new cases and 74,000 deaths each year [3].

Ineffectiveness of Pap test in controlling cervical cancer specially in the developing part of the world, can be explained because of the subjectivity of the test as well as a high false positivity and false negativity rate, both of at least 20% [4,5]. A recent overview of European and North American studies revealed that the sensitivity of cytology for high-grade preinvasive lesions and cervical cancer is on average not higher than about 55% [6]. 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.

Apart from screening the conventional cytological parameters in the cervical smear, micronucleus is yet another parameter to screen , which gives the evidential proof for the cervical cancer. Complementary methods have now been aimed at increasing the sensitivity of screening for cervical cancer , including high-risk HPV testing [7] and micronucleus (MN) identification [7,8]. The presence of micronucleus as a biomarker that has

been successfully used to screen populations at risk of cancer and is a sensitive indicator of genetic damage [9]. Micronuclei are small, additional nuclei formed by the exclusion of chromosomal fragments (clastogenesis) or whole chromosomes that are not incorporated into the main nuclei because of mitotic malfunction (aneugenesis)[10] and is a reflection of chromosomal aberration during cellular mitosis. Their frequency appears to increase in carcinogen exposed tissue long before clinical symptoms are evident. We undertook this study to compare micronucleus count in whole spectrum of cervical lesions reported according to Bethesda system ie normal, inflammatory , abnormal squamous cells of undetermined significance (ASC-US), abnormal squamous cells cannot exclude HSIL (ASC-H), low grade squamous intraepithelial lesion(LSIL), high grade squamous intraepithelial lesion (HSIL) and invasive cancer (IC) .

MATERIALS AND METHODS

In this retrospective study , we have compared the micronucleus score in the whole spectrum of cervical lesions comprising of seven groups ie normal (30) , inflammatory (38), ASC-US (2), ASC-H (2), LSIL(20), HSIL(6), IC (2) over a period of one year ,i.e from June 2015 to June 2016. Histopathological correlation was done only for available cases. Two observer separately counted the number of micro nucleated cells on pap stain per 500 epithelial cells in oil immersion magnification and where expressed as MN score per 500 cells

Inclusion criteria:
1. All the cervical Pap smear received having adequate cellularity will be included in the study.
2. Only cells containing intact nuclei that were not clumped, smeared or overlapped will be included in the analysis.

Exclusion criteria:
1. Degenerated cells, apoptotic cells and cytoplasmic fragments will be exempted from counting and scoring.

Criteria for MN

A micronucleus was determined according to the following criteria: Size less than one-third of the main nucleus, clearly included in the cytoplasm on the same optical plane as the nucleus and distinctly separate from the main nucleus with a similar staining intensity (Figure 1A-G). Cells with double or multiple micronucleus were given a score of 1.After screening by two observers , overall consensus was given .Thus for each smear a total of 1000 cells were counted and the numbers of MNC in each case were expressed per 500 cells (MN score)

STATISTICAL ANALYSIS

Anova and t test were used to compare the difference in MN score in seven groups of cervical lesions.
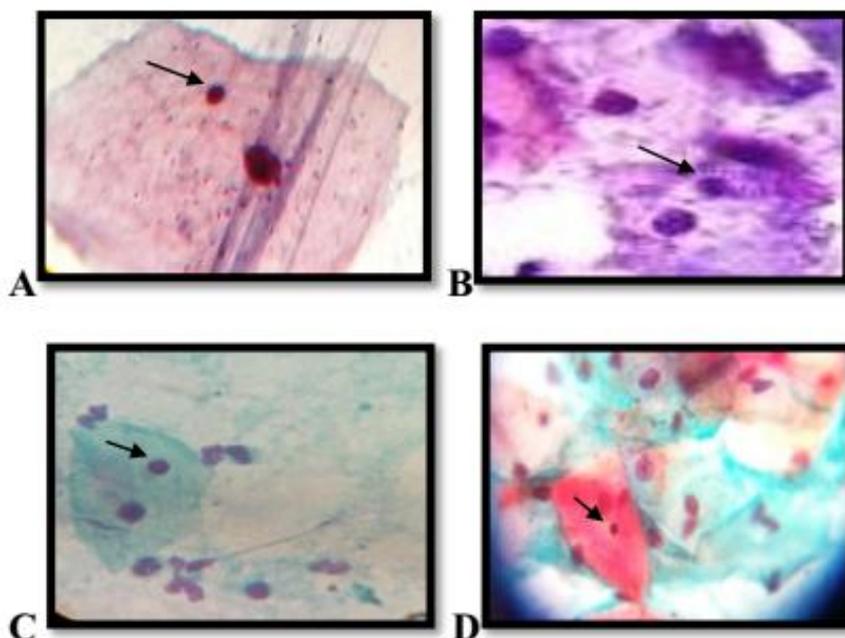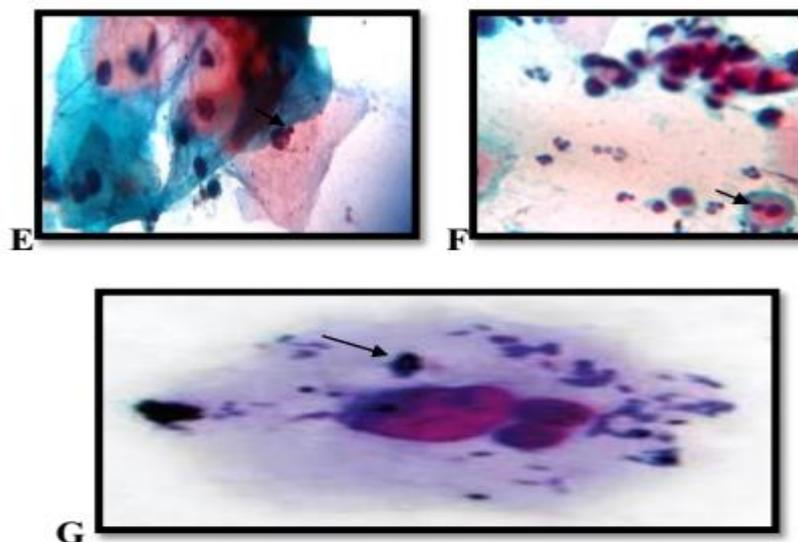
Fig –A (normal), B(inflammatory), C(ASCUS), D(ASCH), E(LSIL), F(HSIL), G(IC).

RESULTS

The mean age of the patients in normal , inflammatory , ASC-US, ASC-H ,LSIL, HSIL and IC cases of cervical lesions are shown in [Table -1]

Among 100 cases , maximum number of cases were inflammatory followed by normal .LSIL was more common in the age group of 31 to 33 yrs with mean age of 44yrs, HSIL was more common in age group of 37 to 53yrs with mean age of 46 yrs and IC was more common in age group of 34 to 56 yrs with mean age of 45 years as shown in Table 1

Table 1- Mean Age

Groups	Number of cases	Age range	Mean age
Normal	30	20-58	39.07
Inflammatory	38	22-68	45.74
ASCUS	2	28-60	44.00
ASCH	2	28-63	45.50
LSIL	20	31-53	44.40
HSIL	6	37-53	46.50
IC	2	34-56	45.00
TOTAL	100		

ASCUS – Abnormal squamous cells of undetermined significance, ASCH: Abnormal squamous cells cannot exclude HSIL, LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, IC: Invasive cancer.

Table 2: Biopsy Outcome

GROUPS	NO OF CASES	BIOPSY OUTCOME
NORMAL	30	NIL
INFLAMMATORY	38	NIL
ASCUS	2	NIL
ASC-H	2	NIL
LSIL	20	14 (CIN I-10,CIN II-4)
HSIL	6	6 (CIN II-2,CIN III-4)
IC	2	2 (SQUAMOUS CELL CARCINOMA).

ASCUS – Abnormal squamous cells of undetermined significance, ASCH: Abnormal squamous cells cannot exclude HSIL, LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, IC: Invasive cancer.

Biopsy outcome was not available in normal. Inflammatory ASCUS ,ASC-H, LSIL cases , however all IC reported on cytology turned out to be squamous cell carcinoma on biopsy as shown in [Table -2]

Table 3: Mean Micro nucleated Score

GROUPS	MN SCORE
NORMAL	2.57 ±1.38
INFLAMMATORY	8.21±1.26
ASCUS	10±2.83
ASC-H	13±2.83
LSIL	15.8±1.44
HSIL	20.33±1.63
IC	23.00±2.83

ASCUS – Abnormal squamous cells of undetermined significance, ASCH: Abnormal squamous cells cannot exclude HSIL, LSIL: Low grade squamous intraepithelial lesion ,HSIL: High grade squamous intraepithelial lesion, IC: Invasive cancer.

The Mean MNC score in cervical lesions are shown in [Table 3]There is stepwise gradual increase in MN Score from inflammatory to ASC-US to LSIL to HSIL group, followed by an increase in IC .[Table 2].The mean MN score was most significant in LSIL and HSIL groups.

The statistical analysis revealed significant difference of MN score in different groups .MN score Of IC and HSIL were significantly high as compared to normal (P<0.0001), inflammatory (P<0.0001), ASC-US(P<0.0007), ASC-H(P=0.0039), LSIL(P<0.0001) groups. LSIL showed significant difference with the normal (P<0.001), inflammatory (P<0.0001), ASC-US(P<0.0001), ASC-H(P=0.0235) HSIL (P<0.0001), IC (P<0.0001).ASC-H showed significant difference with normal (P<0.0001), Inflammatory (P<0.0001),HSIL(P=0.0031), but not with ASC-US (P=0.4002) and IC (P=0.0716) groups. There was a significant difference of MN score noted in normal versus inflammatory(P<0.0001).

DISCUSSION:
Cervical cancer is the result of a long process that has its onset in LSIL and HSIL precancerous lesions. Decades have passed since in-vitro micronucleus assay techniques were first introduced and the theory and significance of MN have also been extensively discussed and well established. But for pathologists the literature on MN on smear preparation is still limited.

MN has generally been used as a biomarker of chromosomal damage, genome Instability and cancer risk, integrating acquired mutations and genetic susceptibility towards mutations [11-14]. The micronucleus test on exfoliated cells has been successfully used to screen population groups at risk for cancers of oral cavity , urinary bladder, cervix and esophagus .The wide variation in micronucleus scores among different individuals in the same group may be attributable to environment exposure, to genotoxic agents , lifestyle factors , micronutrient deficiency , genetic make-up, baseline micronucleus frequency, ethnicity and other factors associated with carcinogenesis and chromosomal damage [15].

In this study , we have done MN scoring in full spectrum of cervical lesion categorized according to Bethesda. We noted significant difference of MN score in HSIL and IC with all other groups. We also noted significant difference of MN score in LSIL and ASC-US with normal and inflammatory lesion. There was no significant difference with ASC-US and ASC-H, also there was no significant difference between ASC-H and IC .

A series of studies conducted by Samanta *et al.;* [15,16,17] , have established the fact that blind MN scoring on cervical exfoliated cells can distinguish between LSIL, and HSIL; ASC-US and LSIL and of course normal/inflammatory versus LSIL, HSIL and IC but not HSIL versus IC or normal/inflammatory versus ASC-US .This was due to the fact that the ASC-US cases were actually inflammatory or metaplastic or atrophic as confirmed on biopsies.

In another study by them on biopsy proven cases of ASCUS with different outcomes, they experienced significant difference in MN scoring in ASCUS with benign outcome versus ASCUS with CIN outcome. Thus, routine MN scoring on ASCUS smears could reduce diagnostic dilemmas and increase the accuracy.

Few previous studies by Patricia Guzma'n *et al.;* [18] did similar kind of study on Pap smears.

They noted that HSIL smears had the highest frequency of micro-nucleated cells (MNCs) [19]. However, the MN frequency for HSIL and LSIL smears were not significantly different in their study.

In study conducted by Campos L *et al.;* [19] exfoliated cervical cells were obtained from patients. The patients age, habits (passive or active smoking, alcoholism and numbers of sexual partners), age at first sexual intercourse, contraceptive methods used, histories of sexually transmitted diseases, use of hormone replacement therapy, numbers of pregnancies and abortions were recorded. The authors concluded that the prevalence of micronuclei in exfoliated cervical cells was greater in patients with one or more risk factors for uterine cervical cancer than in patients without risk factors.

Keratohyaline granules, nuclear debris, bacterial colonies, and stain deposits are the common mimickers of MN , we encountered this problem while counting MN. However MN is non-refractive, round to oval shaped body and the shape, color, and texture of MN are similar to that of the main nucleus .A DNA-specific stain such as Feulgen or acridine orange stain is more specific for MN and can be used .

Singaraju *et al.;* [20] found increased MN frequency in buccal smears of petrol station attendants compared to control group .The MN test in exfoliated epithelial cells seems to be useful marker of occupational exposure to genotoxic chemicals. MN counting may be viewed as a simple and convenient test which can be done easily on conventional smears and serves as an effective biomarker for cervical cancer screening in conjunction with routine pap screening

CONCLUSION

In conclusion, MN scoring is an easy, simple, reliable, reproducible, and objective test and may be helpful in identifying the true CIN cases which are labeled as ASCUS on cervical smear. In future, another new avenue for disease monitoring would be MN scoring on routinely stained smears..

REFERENCES

1. Waggoner SE. Cervical cancer. The Lancet. 2003 Jun 28; 361(9376):2217-25.
2. Valdespino Valdespino V .Cervical Cancer screening: state of the art .Current opinion in Obstetrics and Gynecology.2006 ;18(1):35-40
3. Laikangbam P, Sengupta S, Bhattacharya P, Duttagupta C, DHABALI SINGH T, Verma Y, Roy S, Das R, Mukhopadhyay S. A comparative profile of the prevalence and age distribution of human papillomavirus type 16/18 infections among three states of India with focus on northeast India. International Journal of Gynecological Cancer. 2007 Jan 1; 17(1):107-17.
4. Nanda K, McCrory DC, Myers ER, Bastian LA, Hasselblad V, Hickey JD, Matchar DB. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Annals of internal medicine. 2000 May 16; 132(10):810-9.
5. Fahey MT, Irwig L, Macaskill P. Meta-analysis of Pap test accuracy. American journal of epidemiology. 1995 Apr 1; 141(7):680-9.
6. Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, Szarewski A, Birembaut P, Kulasingam S, Sasieni P, Iftner T. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. International Journal of Cancer. 2006 Sep 1; 119(5):1095-101.
7. Abreu A, Souza R, Gimenes F and Consolaro M. A review of methods for detecting human papillomavirus infection. Virology Journal.2012; 9(1):262.
8. Gayathri BN, Kalyani R, Hemalatha A, Vasavi B. Significance of micronucleus in cervical intraepithelial lesions and carcinoma. Journal of Cytology. 2012 Oct 1; 29(4):236.
9. Samanta S, Dey P. Micronucleus and its applications. Diagnostic cytopathology. 2012 Jan 1; 40(1):84-90.
10. 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. 2011 Jan 1; 26(1):125-32.
11. Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, Fenech M. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. Mutation Research/Reviews in Mutation Research. 2008 Aug 31; 659(1):93-108.
12. Iarmarcovai G, Ceppi M, Botta A, Orsiere T, Bonassi S. Micronuclei frequency in peripheral blood lymphocytes of cancer patients: a meta-analysis. Mutation Research/Reviews in Mutation Research. 2008 Oct 31; 659(3):274-83.
13. Olaharski AJ, Sotelo R, Solorza-Luna G, Gonsebatt ME, Guzman P, Mohar A, Eastmond DA. Tetraploidy and chromosomal instability are early events during cervical carcinogenesis. Carcinogenesis. 2006 Feb 1; 27(2):337-43.
14. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg Å, Pasini B. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series

unselected for family history: a combined analysis of 22 studies. The American Journal of Human Genetics. 2003 May 31; 72(5):1117-30.

15. Samanta S, Dey P, Nijhawan R. Micronucleus in cervical intraepithelial lesions and carcinoma. Acta cytologica. 2010 Nov 26; 55(1):42-7.

16. Samanta S, Dey P, Nijhawan R. The role of micronucleus scoring in fine needle aspirates of ductal carcinoma of the breast. Cytopathology. 2011 Apr 1; 22(2):111-4.

17. Samanta S, Dey P, Gupta N, Mouleeswaran KS, Nijhawan R. Micronucleus in atypical squamous cell of undetermined significance. Diagnostic cytopathology. 2011 Apr 1; 39(4):242-4.

18. Guzmán P, Sotelo-Regil RC, Mohar A, Gonsebatt ME. Positive correlation between the frequency of micronucleated cells and dysplasia in Papanicolaou smears. Environmental and molecular mutagenesis. 2003 Jan 1; 41(5):339-43.

19. Campos LM, Dias FD, Antunes LM, Murta EF. Prevalence of micronuclei in exfoliated uterine cervical cells from patients with risk factors for cervical cancer. Sao Paulo Medical Journal. 2008 Nov; 126(6):323-8.

20. Singaraju M, Singaraju S, Parwani RN, Wanjari SP. Cytogenetic biomonitoring in petrol station attendants: A micronucleus study. Journal of Cytology. 2012 Jan 1; 29(1):1.