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Study of micronuclei in cervical pap smear

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ABSTRACT

Carcinoma of the cervix is a common cause of mortality in women regardless of effective screening methods. Screening for micronuclei (MN) in cells has been one among the tests used in cancer screening. Several micronuclei containing cells rises in carcinogen- exposed tissues much earlier than the symptoms. Here in this study usefulness of micronuclei (MN) in screening cervical lesions was evaluated. We studied comparison of MN score among different cervical lesions and the significance of the difference in the score. We evaluated 406 cervical smears, categorized them using the "Bethesda 2014 system". Mean MN scores among the different cervical lesion categories were compared, and the differences were studied statistically for its significance. Results showed a gradual increase in mean MN score with increasing severity of the cervical lesion and the differences in these scores were statistically significant ($P < 0.05$) among all the categories, with significantly higher mean MN scores in high grade squamous intraepithelial wounds and invasive carcinomas (IC) than others. We concluded that the MN test could serve as an additional and practical screening test in conjunction with the conventional cervical Pap smear for cervical cancer screening.

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INTRODUCTION

Cancer of the cervix is one of the most comprehensively saw causes of mortality among women even though there are preventive measures like screening by cervical Pap smear and HPV vaccination (Aires *et al.*, 2011). After breast and colon cancer, it is the standard wellspring of death worldwide (Adam *et al.*, 2015). In India, approximately 20/100,000 women are likely to suffer from this disease. It is common in the 35-65 years age range (Gayathri *et al.*, 2012).

In 95 to 100% of cases, infection with high-risk strains, i.e. 16 and 18 of Human Papillomavirus is the cause for cervix cancer (Aires *et al.*, 2011; Adam *et al.*, 2015). Presence of koilocytic change in cervical squamous epithelial cells is generally considered as the cytological sign of an HPV infection (Campos *et al.*, 2008).

When detected in the early stages, cervical cancer responds quite well to secondary preventive measures like Pap screening. However, the Papanicolaou test is having a 20% pace of trick negatives and phoney positives, and it isn't appropriate in the finding of cervical injuries. To increase its sensitivity and specificity, the addition of other cytological markers have been suggested. One of them is the MN test which is a cytogenetic method has been proposed (Adam *et al.*, 2015).

Micronuclei are small, additional nuclei that result

from chromosomal aberration (either spontaneous or induced by carcinogens) and failures in the mitotic spindle that lead to the non- incorporation of either chromosome fragments (clastogenesis) or whole chromosomes (neurogenesis) in the cell nucleus. This way, micronuclei address a degree of both chromosome breakage and occurrence and can fill in as a precarious pointer of acquired underhandedness. Before any clinical symptoms appear, their number increases in cells that are exposed to carcinogenic agents (Samanta *et al.*, 2011; Cerqueira *et al.*, 1998). The mean prevalence of MN in the healthy general populations is 0.0 to 0.9% (Campos *et al.*, 2008). As the transformation of CIN Ito CIN III is associated with enhanced genetic instability and genetic damage that is reflected in cytological smears as the appearance of an increased number of MN changes together with an increase in the number of MN may be used as a biomarker in screening cervical smears for risk of developing cancer (Adam *et al.*, 2015). The field of cytogenetic biomarkers is continuously growing and extremely promising (Bonassi *et al.*, 2005).

HPV testing is another complementary method suggested to increase the sensitivity of Pap smears screening; however, being costly, it is not affordable to women having low economic status (Cassel *et al.*, 2014). Some risk factors, like HPV exposure, its oncogenicity, immunodeficiency of patients, along with the existence of co-carcinogens, are associated with cervical cancer (Nwesarfayrouz *et al.*, 2017). Micronuclei prevalence is more in patients having associated risk factors than those having no risk factors (Campos *et al.*, 2008; Bonassi *et al.*, 2005). MN test has been successfully used to screen for cancers of the oral cavity, urinary bladder, and oesophagus as well (Bhat *et al.*, 2016). There are very few studies like ours. We undertook this study to compare MN score in the different categories of cervical lesions and to study its usefulness in assessing the risk of cervix cancer in an early stage, cost-effectively, in a low resource setting and hospitals which cannot afford costlier tests like HPV testing.

Aim

To study the usefulness of micronuclei (MN) score in cervical Pap smear for assessing the risk of cancer

Objectives

1. To review micronuclei score in different categories of cervical lesions by Pap smear
2. To compare micronuclei score in different categories of cervical lesions
3. To evaluate the significance of MN score in assessing cervical cancer risk

Inclusion Criteria

Any age women are coming for Cervical Pap screening.

Exclusion Criteria

1. Pregnant women
2. Women treated for intraepithelial neoplasia
3. Unsatisfactory smears

MATERIALS AND METHODS

The present study was an observational and analytical type. In this study, we examined 406 cervical smears stained with Papanicolau stain received during the period from January 2018 to December 2018.

Cervical cell collection and preparation of Pap smears-by conventional method

Patient's socio-demographic and clinical details were collected from the patient's datasheet. Only after taking the written informed consent from patients, cellular material was obtained on microscopic slides by Gynecologist by shedding of cells from the cervix using Ayre spatula and cervical cytobrush. Two smears were prepared from the cell sample from each patient and fixed in wet condition by immediately dipping it in a fixative which is a mixture of 50% ethanol and 50% ether in Coplin's jar for at least 20 min. After fixation, the smears were stained by Pap's stain and mounted by DPX mountant.

Pap smear reporting

The Pap smears were reported according to standard Bethesda 2014 system as follows Negative for intraepithelial sore or insidiousness, inflammatory,

1. ASC-US, (Atypical Squamous Cells of Undetermined Significance)
2. ASC-H, (Atypical Squamous Cells can't bar HSIL)
3. LSIL, (Low-grade Squamous Intraepithelial Lesion)
4. HSIL, (High-grade Squamous Intraepithelial lesion)
5. IC, (invasive cancer)

Table 1: Distribution of cases according to 'Bethesda system 2014 for cytological reporting of cervical lesions' with their age distribution

Group	No. Of cases (%)	Age range (yrs)	Mean age (yrs)
NILM	140 (34.48)	22-65	38.48
Inflammatory	128 (29.55)	22-60	35.96
ASC-US	36 (8.86)	25-54	36.69
ASC-H	18 (4.43)	36-65	45
LSIL	42 (10.34)	30-71	41.90
HSIL	22 (5.42)	28-65	51.22
IC	20 (4.92)	45-80	61.8
Total	406		

Table 2: Mean micronucleated cell score in cervical lesions

Group	Mean score ± SD	Median (Min-Max)
NILM	0.601 ± 0.246	0.65(0.1-1.1)
Inflammatory	0.826 ± 0.224	0.8 (0.4-1.7)
ASC-US	2.620 ± 0.496	2.575(1.05- 3.6)
ASC-H	4.967 ± 0.970	5.15(3.25- 6.1)
LSIL	3.709 ± 0.674	3.52(2.7-5.1)
HSIL	7.057 ± 1.293	6.95 (4.8 -8.95)
IC	9.855 ± 1.348	9.475(8.1-12.35)

Table 3: Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of means	SE of difference	95% CI	T-Value	Adjusted P - Value
Inflammatory- NILM	0.2250	0.0692	(0.0210, 0.4291)	3.25	0.020
ASC-US – NILM	2.020	0.106	(1.708, 2.331)	9.10	0.000
ASC-H - NILM	4.366	0.142	(3.948, 4.783)	30.81	0.000
LSIL - NILM	3.1084	0.0995	(2.8149, 3.4019)	31.23	0.000
HSIL - NILM	6.456	0.130	(6.073, 6.838)	49.75	0.000
IC - NILM	9.254	0.135	(8.855, 9.653)	68.42	0.000
ASC-US- Inflammatory	1.795	0.107	(1.480, 2.109)	16.81	0.000
ASC-H – Inflammatory	4.140	0.142	(3.721, 4.560)	29.07	0.000
LSIL - Inflammatory	2.883	0.101	(2.587, 3.180)	28.66	0.000
HSIL – Inflammatory	6.231	0.131	(5.846, 6.616)	47.71	0.000
IC - Inflammatory	9.029	0.136	(8.628, 9.430)	66.37	0.000
ASC-H - ASC-US	2.346	0.163	(1.864, 2.827)	14.36	0.000
LSIL - ASC-US	1.089	0.129	(0.710, 1.468)	8.47	0.000
HSIL - ASC-US	4.436	0.153	(3.984, 4.887)	28.97	0.000
IC - ASC-US	7.234	0.158	(6.769, 7.699)	45.84	0.000
LSIL - ASC-H	-1.257	0.159	(-1.727, -0.787)	-7.89	0.000
HSIL - ASC-H	2.090	0.180	(1.560, 2.620)	11.62	0.000
IC - ASC-H	4.888	0.184	(4.346, 5.430)	26.59	0.000
HSIL - LSIL	3.347	0.149	(2.908, 3.786)	22.48	0.000
IC - LSIL	6.145	0.154	(5.692, 6.599)	39.98	0.000
IC - HSIL	2.798	0.175	(2.283, 3.314)	16.01	0.000

Individualconfidence level = 99.66%, Tukey Simultaneous 95% CIs, P -value

Cytogenetic analysis of micronuclei

For this we followed the method as used by Authors

While screening Pap smear simultaneous counting of micronuclei in cells was carried out. Two Pathologists had reported the micronuclei separately and independently. The smears were examined using a binocular light microscope at a magnification of 1000 x (objective = 100 x with eyepiece = 10 x) - oil immersion. Within the smear, only separate cells, without overlapping or folds, were analyzed. One thousand epithelial cells were studied for micronuclei count by each pathologist, using MN identification criteria given below. Average of two of pathologist's observations were taken.

The MN identification criteria followed were –

1. A chromatin structure similar to and colour intensity similar or lighter than that observed in the main nucleus
2. An evident edge, similar to a nuclear membrane
3. A round shape
4. An intracytoplasmic location
5. A diameter < 20% of the nucleus

— Cells with single twofold or diverse MN was given a score of 1.

— The number of MNC (micronucleated cells) in each case was expressed as MN per 1,000 cells.

The association of mean frequencies of micron cleared cells in different cervical lesions.

Categories were studied and compared statistically.

Statistical analysis

Frequency (percentage) of the micronucleated cells (MN %), its mean score in different categories of lesions similarly, Standard deviation were settled. Comparison of mean MN score among different categories of cervical lesions and the significance of the difference between two groups has been tested by using ANOVA, Tukey's pairwise comparison test' and 'Fisher individual tests for differences for means'. When the difference between two means was as P < 0.05, then it was considered as statistically significant

Ethics associated with the study

The Ethical clearance was taken before start study. An informed written consent from participants was taken before enrolment in the study, in English or Marathi (as the case may be).

RESULTS AND DISCUSSION

As shown in Table 1, NILM cases were strikingly more in number and cases of ASC-H were least in number. More number of NILM cases might be attributed to the collection of cases from Papsmearscreening camp along with routine cases in the hospital. In the study by Rathod *et al.* (2016), inflammatory cases were more and HSIL cases were least in number. In contrast, in the review by Navya *et al.* (2016), inflammatory cases were more and ASC-H cases were least in number. As in Table 1, the mean age for NILM, Inflammatory, and ASC-US lesions was less compared to other higher categories. Our findings were similar to that by Gayathri *et al.* (2012) and Rathod *et al.* (2016).

As shown Table 2, there was a gradual increase in mean MN score observed with increasing severity of the cervical lesion from NILM to Inflammatory, ASC-US, ASCH, LSIL, HSIL and IC. As in Table 3, statistical analyses revealed differences of MN score in different groups which were significant statistically as (P <0.05). MN score of IC and HSIL were primarily high as compared to all other groups. There was a significant difference of MN score noted between NILM and inflammatory group (P=0.020) as well. The comparison of findings of MN score in our study with other studies as (Navya *et al.*, 2016), observed very high MN scores in all the lesions compared to that of ours and two other studies mentioned in the table. Our findings were similar to that by Gayathri *et al.* (2012) and Rathod *et al.* (2016).

Adam *et al.* (2015), in their study found that there was a correlation between viral load, micronucleus frequency and grade of the cervical lesion by cytology. There was a significant increase in micronucleated cells in women with a high viral load and a higher degree of the lesion (CIN). In their study, they found few patients despite having low viral load exhibited genomic instability, as demonstrated by a more significant number of micronuclei in the cells and a higher degree of tissue damage (CIN I). These characteristics indicate infection by a more aggressive type of HPV. So, they suggested more frequent follow-up in these patients. In their study, one patient showed no cytological lesions and a low viral load but a high frequency of micronuclei. From this observation, the authors stated that as genomic instability precedes cytological lesions, the patient in question is at high-risk for the progression of the disease and also requires more frequent follow-up. According to them, the characteristics of this patient underscore the importance of micronucleus analysis in combination with the Papanicolau test for the determination of the mounting risk of cervical can-

cer.

Cassel *et al.* (2014) found in their study that although HPV infection is responsible for virtually all cervical cancers, in some situations, the presence of the virus is not linked to the cellular changes visualized by microscopy. Increase in MN score was observed with the presence of HPV-DNA even if the cytology smear was routine; the physician can use this finding as a criterion for more frequent follow-up.

Like other authors (Gayathri *et al.*, 2012) and (Rathod *et al.*, 2016), we also experienced difficulty while counting MN in smears like- nuclear debris, bacterial colonies, Keratohyaline granules and stain deposits which are the common mimickers of MN. A DNA-specific stain such as Feulgen or acridine orange stain is more specific for MN and can be used.

CONCLUSION

It is a simple, rapid, reliable, reproducible, objective, cost-effective test. It can serve as an effective, additional biomarker to be used in combination with the routine, conventional cervical Pap smear screening which can improve the quality of the screening and diagnosis. It can be used as a predictive indicator during the planning and validation of programs for cervical cancer screening and monitoring. However, additional extensive studies are recommended to confirm this.

Source of funding

Self.

Conflict of interest

Non to disclose.

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