

ORIGINAL RESEARCH

Correlation of Visual Inspection of Cervix with Micronucleus Scoring in Urothelial Cells in Intraepithelial Lesions and Cervical Carcinoma in Different Stages

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ABSTRACT

Background: To Study the presence of micronucleus in urothelial cells of patients with normal and abnormal cervical visual inspection and to correlate with cytological and histological findings in normal and various intraepithelial lesions of cervix and cervical carcinoma. To assess the role of urothelial MN score in predicting cervical cancer risk.

Materials and Methods: A total of 350 patients with the complaints of post coital bleed, lower abdominal pain, menstrual irregularities who come in gynaecological out patient department of Rajindra Hospital and Medical College, Patiala were included in our study. Patient's cervix was visually examined, pap smear taken and midstream urine sample collected for MN scoring. Incidence of micronucleus in urothelial cells recorded as micronucleus cells per 1000 cells by 4 cytopathologists.

Results: The mean Urothelial MN score \pm Standard deviation was found to be 2.80 ± 1.58 in inflammatory cases, 4.55 ± 1.63 in ASCUS cases, 5.67 ± 0.58 in ASC- H cases, 5.61 ± 0.50 LSIL cases, 6.09 ± 0.30 in cases of HSIL, 6.50 ± 0.53 in invasive carcinoma. A step-wise increase was observed in MN score from inflammatory to IC categories.

Conclusion: Urothelial MN score is a reliable and easy test that can be used along with routine cervical PAP to assess the risk of premalignant and malignant transformation in the cervix as a biomarker for predicting the risk of carcinoma.

Keywords: MN, ASCUS, ASC-H, LSIL, HSIL, IC.

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INTRODUCTION

Worldwide, cervical cancer ranks fourth amongst all malignancies for women after breast cancer, lung cancer and colorectal cancer.^[1] It is leading cause of mortality amongst deaths due to cancer in developing countries. Cervical cancer is malignant tumour of cervical cells. Human papilloma virus is most common cause of cervical cancer. Risk factors include women with multiple sexual partners, sexual relations at early age, smoking, weak immune

system, first birth at very young age, long term use of oral contraceptives, family history, etc. Early-stage cervical cancer generally produces no signs or symptoms. As compared to other gynecological cancers, precancerous lesions of cervix are detected in younger age groups. Thus screening should be started from early 20's. Cellular damage in exfoliative urothelial cells is observed in patients undergoing cervical cytogenic changes. In developing country like India, test like MN score in urothelial cell which is not too expensive and is easy sampling method can thus be suggested as part of routine screening.

MATERIALS & METHODS

Patients with the complaints of post coital bleed, lower abdominal pain, menstrual irregularities who come in gynaecological out patient department of Rajindra Hospital and Medical College, Patiala were included in our study.

Inclusion Criteria

- Patients whose visual inspection of cervix was normal and whose showed positive findings like erosions on cervix, hypertrophied cervix, abnormal growth, ulcer, or chronic discharge were included in the study.

Exclusion Criteria

- Pregnant women
- Patients suffering from other malignancies.
- Patients on long-term chemotherapy or radiotherapy treatment.
- Patients with urinary tract infection.

Procedure

- After getting the Institutional Ethical Committee acceptance, a Performa was prepared in order to record the history, general examination and pelvic examination.
- After obtaining the consent, patient was visually examined, pap was taken and then requested to collect midstream urine sample (10 ml) in a sterile plastic container (sample to be taken at any time and no medications were recommended before sample collection) and was transported to the laboratory.

The presence of MN was confirmed under oil immersion (100 X), observation was recorded and tabulated. MN cells was recorded in each patient from the slides prepared and incidence of micronucleus was recorded as micronucleus cells per 1000 cells by 4 cytopathologists. The data was collected and analysed using Microsoft Excel Office 2019 and Epi info [CDC Atlanta] vision 7.2.4.0 Most of the data will be expressed in numbers and percentage. Other statistical tests most likely to be use are T test, chi square test, etc keeping in context final distribution and aims and objectives of study.

RESULTS

Table 1 Age and urothelial MN score

Age Group (Years)	0-3		≥4		Total	
	Patients	Percentage	Patients	Percentage	Patients	Percentage
21-35Years	69	37.70%	27	16.17%	96	27.43%
36-50Years	86	46.99%	72	43.11%	158	45.14%
51-65Years	26	14.21%	50	29.94%	76	21.71%
≥66Years	2	1.09%	18	10.78%	20	5.71%
Total	183	100%	167	100%	350	100%

$\chi^2 = 39.345$; p value = 0.001

69 patients between age 21-35 years have MN score 0-3 and only 27 patients between this age group have MN score equal to or more than 4. Whereas patient with age 51 years and above have MN score of equal to or more than 4 in 68 patients and only 28 patients have MN score of 0-3.

Table 2: Follow Up 31 cases of ASCUS in PAP with Urothelial Micronucleus

Follow Up	Patients	MN0-3	MN≥4
Normal	8	7	1
ASCUS	10	0	10
ASCH	5	0	5
LSIL	4	0	4
HSIL	4	0	4

31 patients with ASCUS on pap were followed up after 6 months. Out of 8 Patients who came out to be normal on pap after 6 months 7 had urothelial MN count 0 -3 and only 1 had urothelial MN count ≥4. 13 patients had higher cervical lesion spectrum on pap after follow-up of 6 months and urothelial MN score in all 13 patients were ≥4.

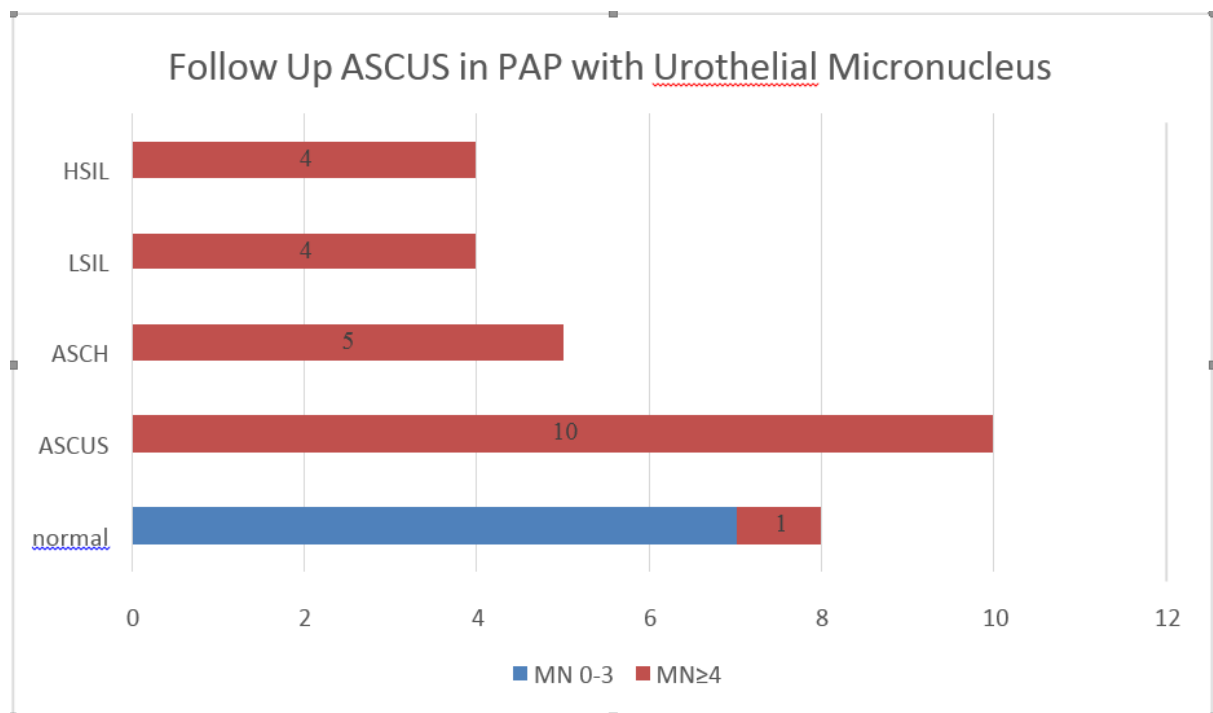


Table3: Cervical Biopsy in already follow up cases of ASCUS

Follow Up	Cervical Biopsy	0-3	≥4	Total
ASCH(N=5)	CIN1	0(0%)	1(20%)	1(20%)
	CIN2	0(0%)	4(80%)	4(80%)
ASCUS(N=10)	CIN1	0(0%)	2(20%)	2(20%)

	CIN2	0(0%)	5(50%)	5(50%)
	CIN3	0(0%)	3(30%)	3(30%)
Low Garde SIL(N=4)	CIN1	0(0%)	2(66.67%)	2(66.67%)
	CIN3	0(0%)	2(33.33%)	1(33.33%)
High Garde SIL(N=4)	CIN1	0(0%)	1(25%)	1(25%)
	CIN2	0(0%)	1(25%)	1(25%)
	CIN3	0(0%)	1(25%)	1(25%)
	Moderate Differentiated Squamous Cell Carcinoma	0(0%)	1(25%)	1(25%)
Normal(N=8)	Chronic Cervicitis	2(25%)	1(12.50%)	3(37.50%)
	Inflammatory Changes	3(37.50%)	0(0%)	3(37.50%)
	CIN1	1(12.50%)	0(0%)	1(12.50%)
	Normal	1(12.50%)	0(0%)	1(12.50%)

Patients with ASCUS on pap were followed up after 6 months. 8 patients after 6 months had normal pap with biopsy finding showing 1 normal, 3 inflammatory, 3 chronic cervicitis and 1 CIN. The comparison with MN count in urothelial cells is made as mentioned above. Repeat pap smear after period of 6 months showed that 10 patients came to be ASCUS, these on biopsy demonstrated different stages of dysplasia categorized under CIN. All these 10 patients had MN count $>/ 4$.

Table 4: Mean micronucleus scoring in urothelial cells in cervical lesions

Group	Mean Score \pm SD	Median (Min.-Max)
Inflammatory	2.80 \pm 1.58	3.00(0-5)
ASC-US	4.55 \pm 1.63	5.00(0-6)
ASC-H	5.67 \pm 0.58	6(5-6)
LSIL	5.61 \pm 0.50	6(5-6)
HSIL	6.09 \pm 0.30	6(6-7)
IC	6.50 \pm 0.53	6.50(6-7)

Table 5: Urothelial MN score with PAP Smear

PAP Smear	0-3(N=183)		≥ 4 (N=167)	
	Patients	Percentage	Patients	Percentage
Normal	13	7.10%	4	2.40%
NILM	95	52.49%	72	43.11%
Atrophic	15	8.20%	7	4.19%
Altered Flora	19	10.38%	6	3.59%
Infection	34	18.58%	9	5.39%
Atypical Glandular Cell	0	0%	3	1.80%
ASCUS	7	3.83%	24	14.37%

ASCH	0	0%	4	1.80%
Low Garde SIL	0	0%	18	10.78%
High Garde SIL	0	0%	10	5.99%
Invasive CA	0	0%	10	5.99%
X ²	59.400			
P value	0.025(S)			

On comparing pap findings with urothelial MN it is observed that as the cervical lesions that are pre-cancerous or cancerous have more patients with MN ≥ 4 as compared to non-cancerous results (normal, NILM, chronic cervicitis, atrophy, altered flora and infections.). It is also observed that chronic cervicitis, although non-cancerous lesion have more number of patient with MN ≥ 4 .

Table 6: Urothelial MN score with Cervical Biopsy

Cervical Biopsy	0-3		≥ 4	
	Patients	Percentage	Patients	Percentage
Normal	21	11.60%	10	5.92%
Squamous metaplasia	2	1.10%	1	0.60%
Atrophic	15	7.73%	2	1.20%
Inflammatory changes	44	21.55%	27	16.07%
Chronic Cervicitis	13	7.18%	24	14.29%
CIN1	1	0.55%	27	16.07%
CIN2	0	0%	20	11.90%
CIN3	0	0%	10	5.95%
Well differentiated squamous cell carcinoma	0	0%	10	5.95%
Moderate Differentiated Squamous Cell Carcinoma	0	0%	7	4.17%
Poor Differentiated Squamous Cell Carcinoma	0	0%	3	1.79%
Adeno carcinoma cervix	0	0%	3	1.79%
Not done	86	47.51%	24	14.29%
Total	182	100%	168	100%
X ²	35.823			
P value	0.020(S)			

out of 350 cases, 110 did not undergo biopsy as elective cases were closed during covid pandemic and few patients did not have indication to undergo biopsy. Study showed, 0-3 MN count maximum in patients with biopsy results showing inflammatory changes. More than or equal to 4 MN score was observed maximum in group of chronic cervicitis and inflammatory changes on biopsy. It was observed that 20 patients showed malignant changes in histopathology and all these patients belonged to group with MN score equal to or more than 4.

DISCUSSION

In developing countries like India, carcinoma cervix remains the major burden. In carcinoma cervix, India stands first among middle eastern countries. Among the women of reproductive age, cervical carcinoma is major cause of cancer. It is therefore important to do timely and efficient screening for adequate prophylaxis and treatment. Cytology has its own limitations with high rates of false negativity. This may be due to variability in samples, preparation of smears, staining techniques and observer bias. Pap smear is highly sensitive therefore used as mass screening tool ,however its specificity is low. This study explores the possibility of using urothelial MN frequency as simple inexpensive screening test for early detection of carcinoma cervix. According to our study as the age increases MN score in urothelial cells increases. This is comparable to Suresh Sundararajan et al where MN score had linear correlation with age. Early age of marriage contributes to

higher MN score in urothelial cells. Urothelial MN score is significantly high in females who got married between 15-19 years as compared to females who got married after 25 years. This is comparable to TM Viny et al and Gandhi et al who also noticed early age at marriage as a significant risk factor for carcinoma and correlated it with elevated MN score in urothelial cells.

Limitations of This Study

The major limitation in study was:

1. Large urine samples.
2. Contaminated urine samples with blood and bacteria.
3. Mimickers such as bacteria, pus cells, clumped cytoplasm in smear.
4. The result of current study should be confirmed with the large size population study.

Summary

1. All 350 patients underwent pap screening and simultaneous urothelial micronucleus assay, whereas only 240 patients underwent cervical biopsy depending upon observations made on visual inspection of cervix.
2. 22.22% patients out of 350 had precancerous and cancerous outcomes on pap screen whereas 48.2% patients had significant MN count. The difference owes to observation of higher MN in urothelial cells due to UTI, chronic cervicitis, making it less specific.
3. Patient whose age at marriage was between 15-19 years were at higher risk for carcinogenesis. Patients with age at marriage after 24years showed less urothelial MN score.
4. There was increase in micronucleus score from inflammatory to chronic cervicitis, ascus, LSIL, HSIL, invasive carcinoma.
5. Amongst pap, 31 patients had ASCUS as outcome along with MN score varying from nil to 6. These patients were closely followed for 6 months and repeat PAP were taken. Patients then underwent biopsy and it was observed that patients with higher urothelial MN score progressed to higher pap outcome after 6 months and even had precancerous or cancerous biopsy outcomes. Whereas patients with ASCUS on first screen and lower MN score (0-3) came out to be normal after 6 months follow up with no significant biopsy findings.
6. Among 350 patients, 243 patients belonged to lower middle socioeconomic status. Amongst these 243, 66.86% had significant MN count. But the MN count in the upper SES was not statistically significant.

7. A linear association was observed between urothelial MN score and cervical lesion spectrum.
8. Sensitivity and specificity of urothelial MN came out to be 90.70 % and 70.65% respectively.
9. The study had Positive Predictive Value of 89.66%; Negative Predictive Value of % 73.03 with Accuracy Value= 85.43.

CONCLUSION

Cellular damage in exfoliative urothelial cells is observed in patients undergoing cervical cytogenic changes. In developing country like India, test like MN score in urothelial cell which is not too expensive and have easy sampling method can be suggested as part of routine screening.

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