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RESEARCH ARTICLE

CONNOTATION OF MICRONUCLEUS AS A BIOMARKER IN CERVICAL INTRAEPITHELIAL LESIONS AND CARCINOMA

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Abstract

Introduction: The Pap smears study is the most rampant and cost-effective method for cervical cancer screening. The role of micronuclei in exfoliated cells of pap smears is the greater beneficial tool used in the screening of cervical cancers. They are intracytoplasmic inclusions made up of chromatin fragments that suggest instability in the chromosomes and they are found in great numbers in carcinogen-exposed tissues long before any clinical signs occur.

Aims: To evaluate the predominance of Micronuclei as a biomarker in various premalignant and malignant lesions of cervixand to explore the association between various grades of cervical lesions and micronuclei frequency.

Materials &Methods:A prospective study was done in the cytopathology section on 250 cervical pap smears collected in the Pathology laboratoryRajarajeswari medical college, Bangalore. The study period was from January 2021 to June 2021. Slides containing the cell material were stained with Papanicolaou stain, The results were analyzed as per the Micronuclei Scoring done per 1000 exfoliated squamous cells under light microscopy

Results: In this study of 250 cervical pap smear screening, there was a stepwise gradual increase in micronuclei count(MN score)from Inflammatory lesions to carcinoma cervix. Inflammatory 2.04 ± 0.43, Atypicalsquamous cells of undetermined significance 3.9 ± 1.73, Atypical squamous cells - High grade5.64 ±1.83,Low grade squamous intraepithelial lesion 7.68 ±1.48, High grade squamous intraepithelial lesion08 12.32 ± 2.68and Intraepithelial Carcinoma 18.60 ± 2.83.

Conclusion: MN scoring on cervix squamous epithelial cells is a simple and reliable objective test that may be performed on routine Pap-stained smears and can be used as adjunctive to the screening of cervical cancers and provide a potential pathway for disease monitoring in the future.

Introduction:-

Cancer cervix is a primary cause of morbidity and mortality among women globally, with more than half a million new cases diagnosed each year. It is therefore a considerable public health issue. The second most prevalent cancer in women is cervix cancer and the most familiar cancer in several developing nations[1].

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Since cervical cancer develops from precursor lesions over time and loses aberrant cells, cytology screening is beneficial in preventing it. Dysplasia of the epithelial or surface lining of the cervix is the first microscopic change associated with CIN.

The Papanicolaou (Pap) test is a well-established procedure for determining the dysplastic cells obtained from pre-neoplastic differentiation of the cervix[2,3].

Another test that has been successfully utilized to screen population groups at risk for malignancies of the oral cavity, urinary bladder, cervix, and oesophagus is the micronucleus (MN) test on exfoliated cells.

Micronuclei are the extra-nuclear remnants formed during the cell division process because of damaged chromosomal fragments that are not integrated into the cell nuclei. The existence of MN indicates genomic instability due to chromosomal aberration and represents cancer risk. In most cancers, the number of micronuclei increases over a while[4].

The target of this study is to look at the prevalence of micronuclei in exfoliated cells of cervix mucosa to see if there is an association between the presence of micronuclei and high-grade cervical lesions and cervical cancer.

MN assay is a fast, safe and a reliable technique to assess genomic instability and does not require too much expensive equipment, this test could be incorporated into routine Pap test an additional criterion for the early detection of genomic instability.[5].

The objective of this study is to analyze the importance of micronuclei in exfoliated cells ofcervical pap smears and to explore the association between various grades of cervical lesions andmicronuclei frequency. To evaluate the predominance of Micronuclei as a biomarker in variouspremalignant and malignant lesions of cervix.

MN score may be helpful in identifying the true CIN cases that are mislabelled as ASCUS on cervical smear. In future, MN score can be used as an additional biomarker in cervical cancer screening[6].

Materials & Methods:-

Source of Data:

This was a 6-month prospective study of the MN score in the entire spectrum of cervical lesions which was performed in the Department of Pathology, Rajarajeswari medical college, and hospital (January 2021 to June 2021).IEC NO: RRMCH-IEC/51/2021

A total of 250 cases were included in this investigation, with normal (52), Inflammatory (158)and Atypical squamous cells of undetermined significance (ASCUS) cases were 18

Atypical squamous cells – High grade(ASC-H) -09 cases and Low grade squamous intraepithelial lesion (LSIL) were 03 in the study

There were 8 cases of High grade squamous intraepithelial lesion (HSIL) 08 and Intraepithelial Carcinoma (IC)were only 02 cases.

Inclusion Criteria:

1. The study included all cervical pap smears with adequate cellularity.
2. The study included only cells with the presence of complete nuclei that are not clumped, smeared, or overlapped.

Exclusion Criteria:

1. Reporting on insufficient samples.
2. Counting and scoring was not done on degenerated cells, cells obscured by blood, debris, mucus, inflammatory cells and bacteria

Methodology:-

Exfoliated cervical cells were collected with the sterile cytobrush and Ayre's spatula by gynecologists after obtaining consent from patients that these samples will be examined for study purposes.

Smears were prepared from each sample on a glass slide, which was fixed in 95% alcohol for 10-15 minutes and stained with conventional Papanicolaou stain in the department of cytology.

A prospective study was conducted from January 2021 to June 2021 in the department of pathology on 250 cervical PAP smears which were classified according to Bethesda 2015 guidelines for reporting cervical cytology. [7]

Criteria for Micronuclei:

- The diameter of MN was variable from 1/16 to 1/3 the diameter of the main nucleus.
- The shape, color, and texture of MN were the same as those of the nucleus.
- Staining intensity was similar to, or slightly weaker than that of the nucleus.
- Round to oval in shape having proximity but no actual contact with the nucleus.
- The plane of focus was the same as that of the main nucleus.
- Cells lying singly were preferred.

MN scoring:Counting of micronuclei per 1000 epithelial cells under oil immersion(x100). The zigzag method was followed for screening the slides using Tolbert et.al criteria of micronuclei scoring [8].

The significance of variance between the mean MN scores of the diagnostic groups was analyzed by one-way analysis of variance (ANOVA). The least significant difference/least square deviation test was used to calculate the P value.

Results:-

Out of the 250 cases, the majority, 158(63%) cases were inflammatory while 52 (21%) cases were of NILM, 18 (7%) cases were of ASCUS, 09 (4%) cases were ASCH, 03 (1%) cases were LSIL, 8 (3%) cases were HSIL, and 02 (1%) cases were of invasive carcinoma (IC). The inflammatory lesions and Negative intraepithelial lesions were included under non-neoplastic cervical lesions. LSIL, HSIL, and IC lesions were covered under neoplastic lesions and ASCUS cases were categorized separately.

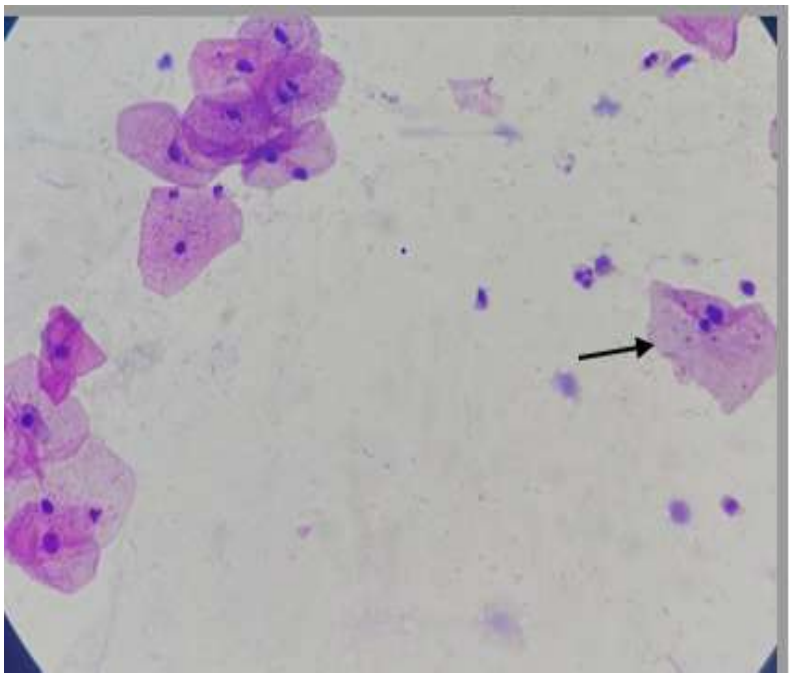

Fig1:- 10X Pap stain: Inflammatory smears showing showing micronuclei.

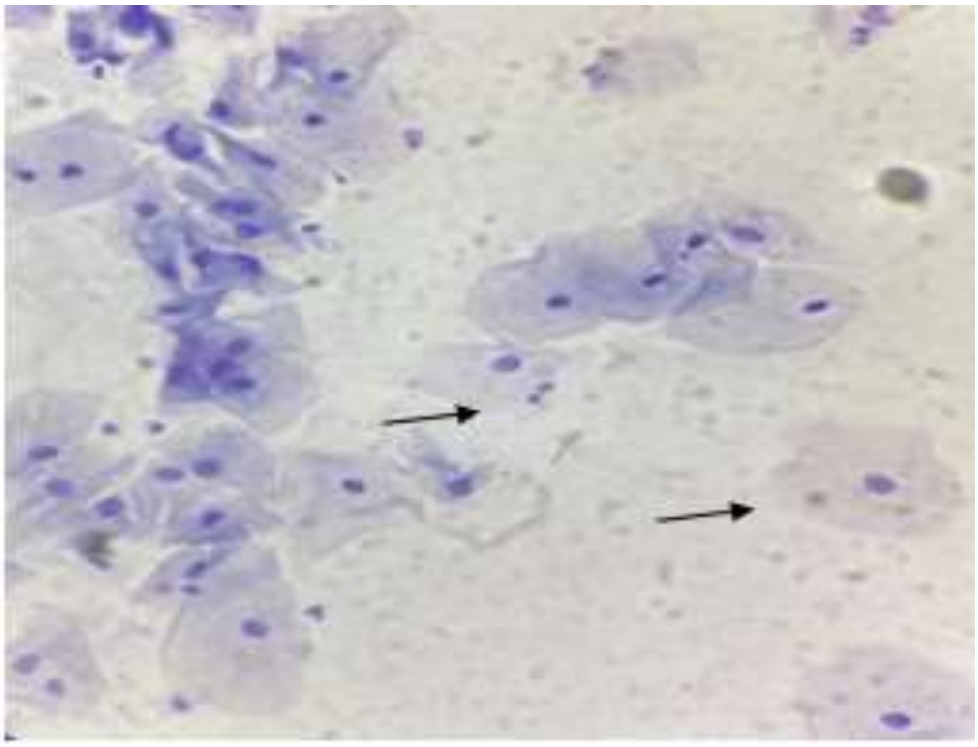

Fig 2:- 10X Pap stain: ASCUS smear showing micronuclei.

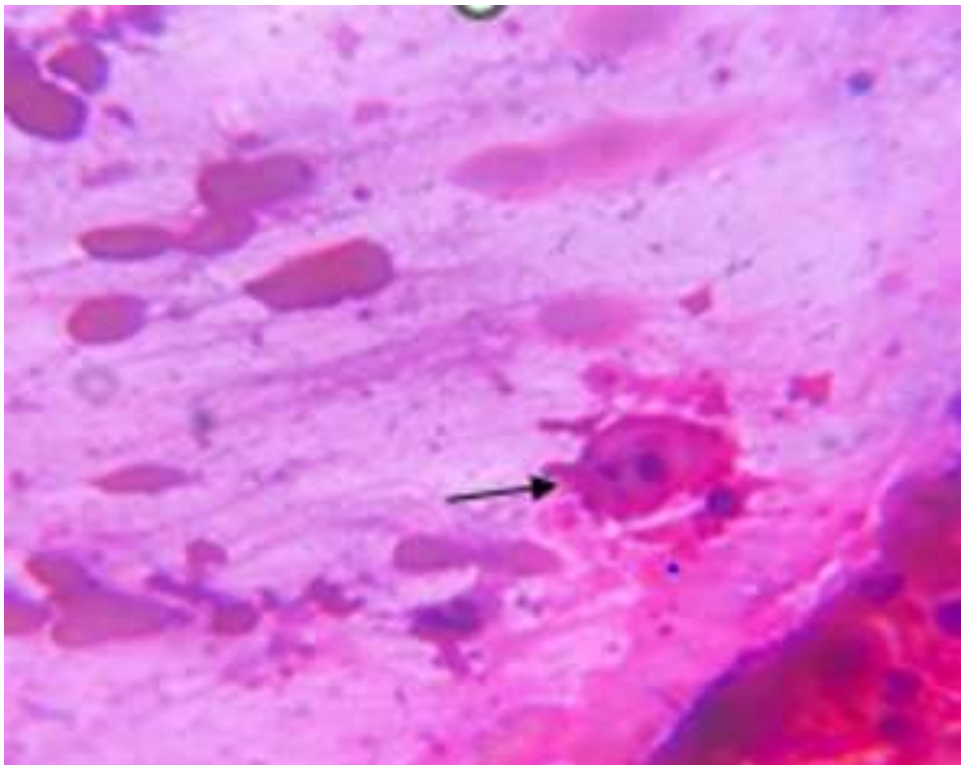

Fig 3:- 10X Pap stain: ASC-H smear showing micronuclei.

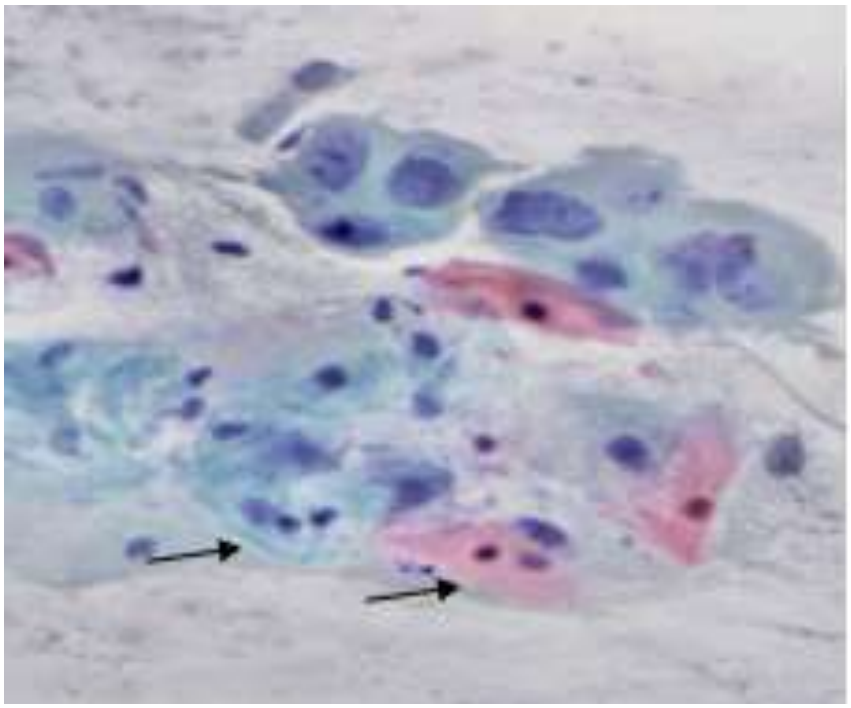

Fig 4:- 10X Pap stain: LSILsmear showing showing micronucleimicronuclei.

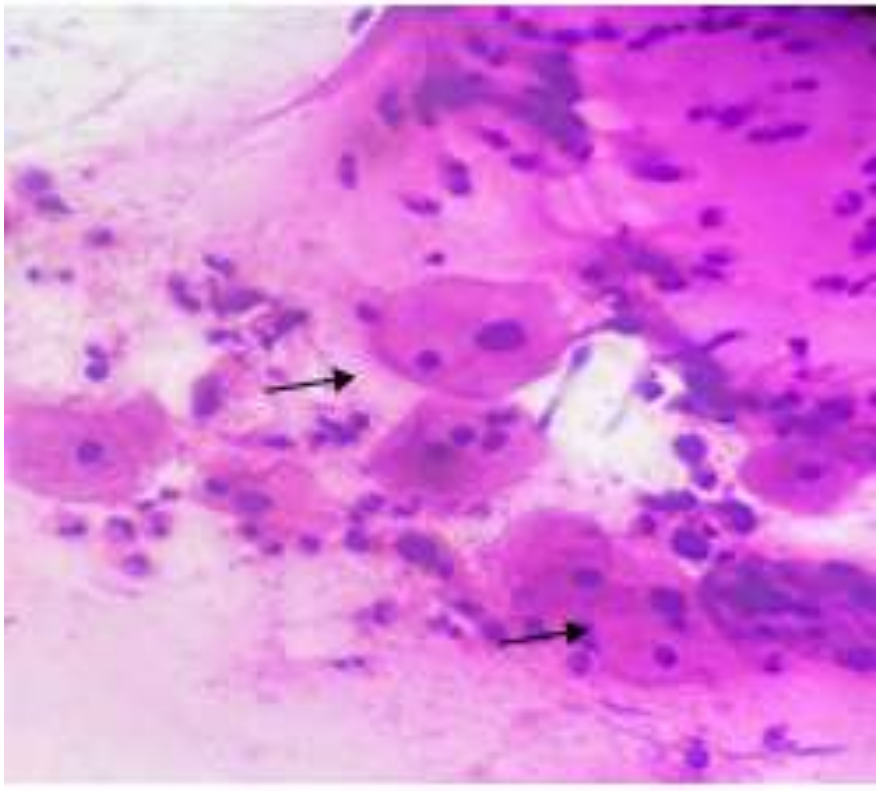

Fig 5:- 10X Pap stain:HSIL smear showing micronuclei.

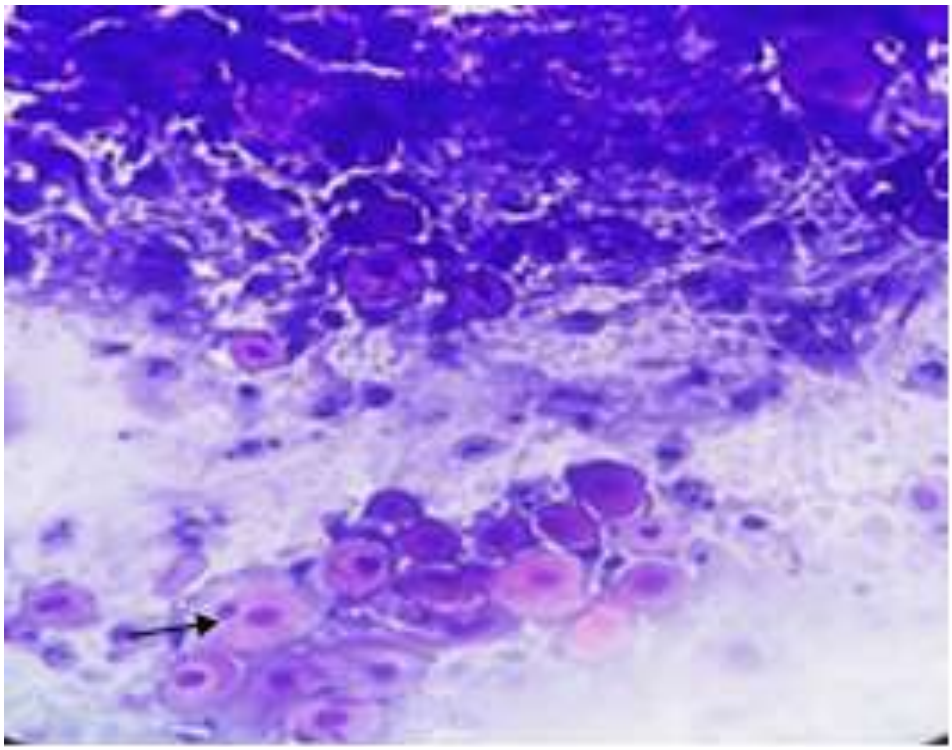

Fig 6:- 10X Pap stain: Intraepithelial Carcinomamicronuclei.

Table 1:- Mean age distribution of the patients.

Groups	**Number of cases**	**Age Range in Years**	**Mean Age in Years**
Normal	52	28-37	32.50
Inflammatory	158	27-38	32.50
ASCUS	18	28-60	44.00
ASCH	09	28-64	46.00
LSIL	03	33-55	44.00
HSIL	08	37-53	45.00
IC	02	34-56	45.00
TOTAL	**250**		

Atypical squamous cells of undetermined significance (ASCUS), Atypical squamous cells – High grade (ASC-H), Low grade squamous intraepithelial lesion (LSIL), High grade squamous intraepithelial lesion (HSIL) and Intraepithelial Carcinoma (IC).

The mean age was more in ACSH, HISL, and IC groups compared to NILM and inflammatory groups.

Table 2:- Micronuclei score in various cervical pap smear lesions.

Groups	**MN Score**	Mean sum of squares	P Value
Normal	1.80 ± 0.58		
Inflammatory	2.04 ± 0.43		
Atypical squamous cells of undetermined significance (ASCUS)	3.9 ± 1.73		
Atypical squamous cells – High grade (ASC-H)	5.64 ±1.83		
Low grade squamous intraepithelial lesion (LSIL)	7.68 ±1.48		
High grade squamous intraepithelial lesion (HSIL)	12.32 ± 2.68		
Intraepithelial Carcinoma (IC)	18.60 ± 2.83	293.983	<0.0001

The mean MN scores ± SD in NILM, inflammatory, ASC-US, ASCH, LSIL, HSIL, and SCC cases were1.80±0.58, 2.04±0.43, 3.9±1.73, 5.64±1.83,7.68±1.48, 12.32±2.68, and 18.60±2.83 respectively.

Micronucleus in various lesions is depicted in the images shows a stepwise gradual increase in MN score from inflammatory to ASC-US to LSIL to HSIL group, followed by a slight increase in IC. The result was statically significant with p value <0.0001

Table 3:- Biopsy outcome.

Groups	Number of cases	Biopsy Outcome
Normal	52	Biopsy was not done
Inflammatory	158	Biopsy was not done
Atypical squamous cells of undetermined significance (ASCUS)	18	15 cases- Chronic cervicitis with koilocytosisand 3 cases- CIN I
Atypical squamous cells – High grade (ASC-H)	09	2 cases - Chronic cervicitis, 6 cases- CIN I and 1case - CIN II
Low grade squamous intraepithelial lesion (LSIL)	03	1 case - CIN I and 2 cases - CIN II
High grade squamous intraepithelial lesion (HSIL)	08	5 cases- CIN II and 3 cases - CIN III
Intraepithelial Carcinoma (IC)	02	2-Squamous cell carcinoma cervix

CIN- Cervical Intraepithelial Neoplasia

Discussion:-

The test for micronucleus was first proposed in the 1970s by Boller and Schmidt. In 1982 Stich and his colleagues stated the human biomonitoring study by Micronuclei procedure in exfoliated cells of buccal mucosa and other tissues like the oesophagus [9].

The micronuclei scoring is considered an elementary, diligent, modest, feasible, and non-invasive test used for treating patients who are at high carcinogenic risk and vulnerability to genotoxic agents such as tobacco, radiation exposure, and oncogenic virus. The prevalence of micronuclei in clinically normal tissue, in high-risk patients is analogous to a higher risk of carcinogenesis.[10].

Micronucleus is shaped by Chromosomal particles which abort to integrate into the cell during cell division. Micronucleus formation is a precise biomarker for chromosomal destruction and indicated the deterioration of chromosomes [11].

This study included the complete spectrum of cervical lesions, which showed a compelling difference in micronuclei score in High grade squamous intraepithelial lesions and intraepithelial carcinoma when compared to low-grade lesions and other inflammatory lesions[12].
The ample discrepancy in the Micronuclei scores among the similar group but different individuals may be likely due to genotoxic agents exposure, variable lifestyle modifications, genetic factors, different ethnic groups, and various other elements related to carcinogenesis and breakdown of chromosomes.
These factors recommended that micronuclei formation is not solely involved in carcinogenesis, relatively it is considered a multifactorial phenomenon. The marked increase in micronuclei frequency is further reminiscent of heightened chromosomal damage rather than cancer. However, malignant and premalignant settings show significant micronuclei score. As a result, increased MN is indicative but not diagnostic of pre-neoplastic conditions, and caution should be exercised through careful clinical history and examination [13,14].

The main components that promote the micronuclei formation are metabolic stress by the neoplastic growth, the products released by the tumor which are clastogenic, mitosis dysfunction, anaphase bridges breakdown and a high risk of HPV infection [15].

The micronuclei scoring is a simple, fast, and predictable test done on conventional pap smears cervical epithelial cells and acts as a biomarker in cervical cancer screening and early revelation of genomic instability. The difficulties endured during micronuclei scoring are bacterial colonies, keratohyalin granules, nuclear debris and stain deposits [16,17,18].

Table 4:- Comparison of Micronuclei score in various studies and present study.

	Present study	Kalpana MK et al [30]	Gayathri et al[1]	Samantha et al[2]	Ambrosie et al[6]
NILM	1.80 ±0.58	0.38 ±0.7	0.84 ±0.6	1.02 ±1.5	1.2 ±1.1
Inflammatory	2.04 ±0.43	1.04 ±1.0	1.06 ±0.8	0.42 ±0.7	2.5 ±1.4
ASCUS	3.9 ±1.73	1.29 ±0.5	3.00 ±0.7	2.87 ±2.22	3.4 ±1.4
L-SIL	7.68 ±1.48	1.89 ±0.6	4.77 ±1.4	4.73 ±5.6	4.21 ±0.97
H-SIL	12.32 ±2.68	3.73 ±1.9	4.77 ±1.4	21.3 ±17.1	4.86 ±1.21
Intraepithelial Carcinoma (IC)	18.60 ± 2.83	6.67±0.81	10.5±2.01	18.50 ± 9.54	5.78 ± 8.6

Atypical squamous cells of undetermined significance (ASCUS), Atypical squamous cells – High grade (ASC-H), Low grade squamous intraepithelial lesion (LSIL), High grade squamous intraepithelial lesion (HSIL) and Intraepithelial Carcinoma (IC).

The Present study was similar to Kalpana M K et al and Gayathri et al studies showed significant difference with LSIL thannormal ($p<0.000$), inflammatory ($p=0.001$), ASC-US ($P=0.028$), HSIL ($p<0.000$), and IC ($p <0.000$), but not with the ASC-H ($p=0.64$) group.

Samantha et al and Ambrosie et al study stated that MN scores of IC and HSIL were significantly high compared to the normal ($p < 0.000$). They also found that a gradual increment of MN scores with an upward trend was noted from the NILM to LSIL. A huge leap was evidenced from LSIL to HSIL, as observed in the current study.

Safioz et al. stated that bacterial colonies are discriminated from micronuclei by their peculiar color, shape, and depth of staining. Deposits of stain are seen as polymorphic granules on the cells and are easily distinguished from clearly stained micronuclei in Pap stain [19].

Palaskar et.al has demonstrated that, compared to Giemsa stain, Pap stain is preferable for Micronuclei counting because in the clear cytoplasm, micronuclei are identified conveniently.[20]. Liquid-based cytology smears are better for the micronuclei counting and for special DNA -specific special stains as an access to genomic damage encountered in exfoliated cervical epithelial cells[21].

Micronuclei counting in exfoliated cells with intact boundaries evaluated by various criteria described by Heddle, Stich and Rosin, and Tolbert etal criteria for revealing the micronuclei as follows [22].

In the present study of 250 Cervical Pap smear screening there was a stepwise gradual increase in micronucleus count from inflammatory to Abnormal squamous cells of Undetermined significance to Abnormal squamous cells cannot exclude HSIL to Low grade squamous intraepithelial lesion to High grade squamous intraepithelial lesion and IC group [23,24].

Therefore, the merger of micronuclei assays and pap smear tests is an adjuvant to increase sensitivity and specificity. Genomic insult plays a pivotal role in the progression of degenerative disorders and cancers MN surveillance could be a screening method in routine pap smear reporting for prior identification of cytogenetic aberrations in the assessment of cancer [25,26].

Limitations of the study may be small sample size and in conventional pap smear study, false positive results are higher due to presence of nuclear debris and stain deposits.

Conclusion:-

The micronuclei scoring is a simple, fast, and predictable test done on conventional pap smears, cervical epithelial cells, and acts as a biomarker in cervical cancer screening and early revelation of genomic instability

This Micronuclei scoring could be incorporated into routine Pap tests as an additional criterion for the early detection of genomic instability since it is fast, safe, and reliable and does not require an expensive equipment.

As a result, combining the MN assay with the regular cervical pap smear reporting will improve the sensitivity and specificity by providing information on genomic aberrations as well as cytological parameter evaluation.

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Conflict Of Interest:
The authors declare no conflict of interest

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