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Significance of Micronucleus in Cervical Smears – Pilot Study

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Abstract: ***Aim: Evaluation of the significance of micronuclei (MN) as biomarkers for evaluation the risk of malignant transformation in uterine cervix. MN are intracytoplasmic inclusion bodies from chromatin fragments or whole chromosomes. Their presence in cells is a reflection of chromosomal aberration during cellular mitosis. Patients and methods: MN screening was done in all selected cytopathological smears (conventional Papanicolaou test) by counting 1000 cervical squamous cells with a light microscope at a magnification of 1000x. Results: Comparisons between women with progressive increases in cervical intraepithelial neoplasia (CIN) and control group. The MN frequencies observed were significantly higher in the groups with cellular changes compared to the control group. Conclusion: The results described that the MN test in cervix smears could be incorporated into routine screening procedures as an additional criterion for early detection of cytopathologocal damage. However, additional detailed, systematic studies are needed to confirm this suggestion.***

Keywords: Micronuclei, Papanicolaou test, cervical smears

1. Introduction

Cervical cancer is one of the most frequent female cancers. The estimated worldwide incidence of cervical cancer is approximately 500,000 new cases per year, and the overall five-year survival rate is in the range of 44 to 66% for all clinical stages [1].

Since most cancers arise in epithelial tissues, exfoliated epithelial cells may be particularly useful for monitoring patients who are exposed to risk factors . Epidemiological evidence indicates that in most cervical cancer patients, squamous cell carcinoma is the predominant histological type [2]. This carcinoma results from progression of preinvasive cervical intraepithelial neoplasia (CIN) grade I to CIN III. CIN is the potentially premalignant transformation and abnormal growth (dysplasia) of squamous cells on the surface of the cervix [3]. CIN is not a cancer, and is usually curable.

The earliest microscopic change corresponding to CIN is dysplasia of the epithelial or surface lining of the cervix, which is essentially undetectable by the woman. Abnormal cells are cells which look more or less deviate from the normal appearance, and this variation include inequalities in shape and size not only cells but also their nuclei, a disorder of maturation and relations cytoplasm/nucleus, varying amounts and quality of chromatin, appearance and number nucleoli, presence of mitosis, as well as the way the orientation of nuclei. Not all levels of CIN mandatory and premalignant lesions; in premalignant lesions we include only severe dysplasia CIN3. CIN is estimated at 2-5% of all women, and the malignant potential of 10 - 15% [4].

The Papanicolaou test (Pap smear) is the main measure for the prevention of this type of cancer and is capable of detecting pre-invasive lesions in the slow progression of the tumor [5]. Another test is the micronucleus (MN) test on exfoliated cells and it has been successfully used to screen population groups at risk for cancers of oral cavity, urinary bladder, cervix and esophagus [6]. MN are intracytoplasmic inclusion bodies derived from chromatin fragments or whole chromosomes; their presence in cells is a reflection of chromosomal aberration during cellular mitosis. Their frequency appears to increase in carcinogen-exposed tissues long before any clinical symptoms are evident.

Several studies have indicated that this test can be used as an auxiliary measure for biologically monitoring women who are at risk of developing cervical cancer [7].

The aim of this pilot study was to evaluate the significance of the micronucleus presence in cervical smears in correlation with the age of patients.

2. Materials and Methods

Samples were obtained from 40 female patients between 19 and 78 years of age. Cytological samples were obtained through the conventional Papanicolaou method.

The material for the analysis of micronucleus frequency was obtained using the method employed for the Papanicolaou test. Slides containing the cell material were stained with Giemsa, remaining immersed in the stain for five minutes. The slides were then rinsed with distilled water, left to dry at room temperature and analyzed under an optical microscope, with 1,000 cells examined per patient. Micronuclei were determined according to the following: 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.

3. Results

This pilot study included 20 women with cervical intraepithelial neoplasia grade I (CINI) at the baseline visit and 20 healthy controls. All patients with CIN I had a follow up visit in 2015 when CIN II is diagnosed.

The micronucleus analysis revealed differences in the number of micronuclei found in the groups which ranged from 0.007 to 0.038 among women with CIN I and 0.004 to 0.012 in the control group. There wasn't statistical significant difference in the frequency of micronucleated cells between groups.

In 2015, subjects with previous CIN I were confirmed as CIN grade II patients. The micronucleus analysis revealed differences in the number of micronuclei found in the groups which ranged from 0.013 to 0.037 and comparing to a control group no statistical significant difference in the frequency of micronucleated cells between groups was found.

Spearman's correlation coefficients didn't show significant correlation between micronucleus frequency and age of patients either with CIN I (r=0.079, p=0.742) or CIN II (r=-0.100, p=0.674).

4. Discussion

Genome instability is considered as a predisposing factor and one of the primary events in a malignant transformation [8]. Our preliminary results indicate that micronucleus frequency as a marker of genome instability cannot be used as the only predictor for the evaluation of the risk of malignant transformation in uterine cervix. It's important biological marker in the diagnosis, prognosis and risk assessment of a disease. However, our study didn't show it as a capable in differentiating groups.

Other studies with bigger sample have shown significant difference between healthy controls and groups of women with CIN I or CIN II. Pandey *et al*. [9] recommends micronucleus assay as a triage tool for borderline cases of cervical dysplasia. They included 169 samples for final analysis and their results shows that MN test can be a helpful screening tool together with conventional Papanicolau test for screening of cervical cancer.

Gayathri *et al*. [10] compared the MN score in the whole spectrum of cervical lesions. They analyzed 221 slides in total and confirmed significant difference of MN score between group of patients with normal finding compared to those with inflammation, low-grade squamous intraepithelial lesion or invasive cancer.

Our study has some limitations. It was conducted on the relatively small study population and didn't analyze any risk factor such as positive family history, smoking status, exposure to genotoxic agents, lifestyle factors that could impact number of micronucleated cells. Previous studies shown that patients with one or more risk factors for malignant transformation in uterine cervix have greater prevalence of MN compared to the patients without risk factors [11,12].

More comprehensive approach would include larger study population and analysis of the risk factor impact on the test results.

5. Conclusion

Micronucleus assay as an methodological simple, cheap, reproducible objective test should be used in cervical cancer screening routine but only in combination with conventional Pap-stained smears. It is necessary to conduct larger, prospective studies to analyze more co-factors impacting the number of micronucleated cells.

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