Chromosomal instability in peripheral blood lymphocytes of endometrial cancer patients: impact of clinical and reproductive factors

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DOI: 10.46793/ICCBIKG25.253M

Abstract: Endometrial cancer (EC) is one of the most common invasive gynecological malignancies in developed countries. In Serbia, it ranks as the sixth most frequent cancer among women, with approximately 700 new cases diagnosed each year. The objective of this research was to investigate chromosomal instability in peripheral blood lymphocytes (PBLs) of patients with EC in relation to potential risk factors. The study included 60 participants, including 30 patients with newly diagnosed EC (mean age 67.77 ± 7.99 years) and 30 healthy control women (mean age 60.23 ± 11.55 years). Chromosomal instability was assessed using the cytokinesis-block micronucleus (CBMN) assay in cultured PBLs. The results showed that cancer patients had a significantly increased average frequency of micronuclei (MN), and nuclear buds (NBUDs) compared to healthy controls (MN: 18.20 ± 2.50 vs. 8.33 ± 1.83; NBUDs: 1.00 ± 1.20 vs. 0.10 ± 0.31; $p < 0.0005$). The average frequency of nucleoplasmic bridges (NPBs) and the nuclear division index (NDI) did not show significant differences between groups (NPBs: 0.03 ± 0.18 vs. 0.00 ± 0.00; NDI: 1.52 ± 0.20 vs. 1.55 ± 0.15; $p > 0.05$). Linear regression analysis revealed that health status (diagnosis), histopathological tumor grade, body mass index (BMI), and number of pregnancies significantly influenced the level of chromosomal instability ($p < 0.0005$), whereas age, smoking habits, place of residence, and history of induced or spontaneous abortions were not significantly associated. These findings suggest that patients with EC exhibit increased chromosomal instability in PBLs, evidenced by elevated frequencies of MN and NBUDs. This instability is significantly associated with clinical and reproductive risk factors, such as tumor grade, BMI, and number of pregnancies, highlighting its potential value as a biomarker for disease monitoring and prognosis.

Keywords: endometrial cancer, peripheral blood lymphocytes, chromosomal instability.

1. Introduction

Endometrial cancer (EC) is among the most common invasive gynecological malignancies in developed countries. While EC is usually diagnosed early and has a favorable prognosis, increasing incidence and mortality underscore the need for better understanding of its molecular mechanisms. Chromosomal instability characterized by increased chromosomal alterations during cell division, plays a central role in tumor progression [1]. Although primarily studied in tumor tissues, chromosomal instability can also be detected in peripheral blood lymphocytes (PBLs) [2].

The cytokinesis-block micronucleus (CBMN) assay is a validated and sensitive method to assess chromosomal damage by scoring micronuclei (MN), nucleoplasmic bridges (NPBs), and nuclear buds (NBUDs) [3]. Preliminary studies suggest that women with EC may exhibit increased genomic instability compared to healthy individuals [4].

This study aimed to evaluate chromosomal instability in PBLs of EC patients using the CBMN assay and to analyze association with clinical and reproductive risk factors.

2. Methodology

Patients

The study included 60 women: 30 with newly diagnosed, histologically confirmed EC (mean age 67.77 ± 7.99 years) and 30 healthy controls (mean age 60.23 ± 11.55 years). Peripheral blood was collected by venipuncture in heparinized syringes. All participants gave written informed consent, and the study was approved by the Ethics Committee of the General Hospital in Kraljevo (No. 20-6), in accordance with the Declaration of Helsinki.

CBMN assay

The CBMN assay was performed using established protocols for genotoxicity testing, with minor modifications [5]. Heparinized whole blood (0.5 mL) was cultured in RPMI-1640 medium with supplements and phytohemagglutinin. After 44 hours, cytochalasin B (4 μg/mL) was added. At 72 hours, cells were harvested, treated with hypotonic KCl, fixed in methanol:acetic acid (3:1), and stained with 2% Giemsa. Micronuclei were scored in 1000 binucleated cells per subject. The nuclear division index (NDI) was calculated based on 500 cells using a standard formula [5]:

$$\mathrm{NDI} = \frac{([1 \times \mathrm{M1}] + [2 \times \mathrm{M2}] + [3 \times \mathrm{M3}] + [4 \times \mathrm{M4}])}{\mathrm{N}}$$

where M1–M4 represent the number of cells with 1 to 4 nuclei and N is the total number of the cells scored.

Statistical Analysis

Data were analyzed using SPSS (version 20). Results are shown as mean ± standard deviation. Group differences were tested with the Student's *t*-test. Linear regression

identified associations between cytogenetic markers and clinical/reproductive factors. A p-value < 0.0005 was considered statistically significant.

3. Results and Discussion

The results of the CBMN assay revealed a significantly increased frequency of chromosomal damage markers in patients with EC compared to healthy controls (Table 1). Specifically, the mean frequency of MN was more than twice as high in patients than in controls ($p < 0.0005$). A similar pattern was observed for NBUDs, with significantly elevated values in the patient group ($p < 0.0005$). However, no statistically significant differences were found in the frequencies of NPBs or in the nuclear division index (NDI) between the two groups.

Table 1. Frequency of cytogenetic markers and nuclear division index in patients with endometrial cancer and control group.

	Patients (X ± SD)	Controls (X ± SD)	p value
MN/1000 BN	18.20 ± 2.50*	8.33 ± 1.83	**0.000**
NBUD/1000 BN	1.00 ± 1.20*	0.10 ± 0.31	**0.000**
NPB/1000 BN	0.33 ± 0.18	0.00 ± 0.00	0.326
NDI	1,52 ± 0.20	1.56 ± 0.15	0.418

* Statistically significant compared to the control group ($p < 0.0005$).

These findings indicate that chromosomal instability is markedly increased in PBLs of EC patients. The elevated MN and NBUDs frequencies are consistent with previous reports highlighting systemic genotoxic damage in cancer patients, including those with gynecologic malignancies [4, 6]. MN reflect both clastogenic and aneugenic events, whereas NBUDs are indicative of gene amplification or unresolved DNA repair intermediates [5].

Table 2. Linear regression analysis of MN, NBUD, NPB, and NDI in relation to the examined variables.

	Model	**MN**		**NBUD**		**NPB**		**NDI**	
		t	p	t	p	t	p	t	p
Linear regression analysis	(Constant)	1.712	0.093	1.350	0.183	-0.049	0.961	6.514	0.000
	Health status	7.037	**0.000**	-0.046	0.964	0.018	0.986	-0.789	0.434
	Age	-0.501	0.619	-0.959	0.342	0.132	0.896	-1.214	0.230
	Smoking habits	-0.491	0.626	-0.974	0.335	-0.278	0.782	0.943	0.350
	Residence	0.417	0.679	-0.534	0.596	0.901	0.372	0.678	0.501
	BMI	2.774	**0.008**	-0.582	0.563	-0.769	0.445	1.579	0.120
	Grade of cancer	0.209	0.835	2.013	**0.049**	0.043	0.966	0.460	0.648
	Number of pregnancies	0.363	0.718	0.597	0.553	-0.080	0.936	2.078	**0.043**
	Number of abortions	-0.440	0.662	-0.393	0.696	1.542	0.129	-1.933	0.059

Linear regression analysis (Table 2) confirmed that health status was a strong and statistically significant predictor of MN frequency ($p = 0.000$), further validating the

utility of CBMN assay in detecting cancer-related genomic instability [5]. Interestingly, BMI also showed a significant positive association with MN frequency (p = 0.008), suggesting that obesity-related mechanisms, such as chronic inflammation, oxidative stress, or estrogen imbalance—may contribute to DNA damage and chromosomal instability [1]. In contrast, the frequency of NBUDs was significantly associated with tumor grade (p = 0.049), implying a potential link between the degree of tumor differentiation and systemic chromosomal instability. The number of pregnancies was also identified as a significant predictor of NDI (p = 0.043), possibly reflecting long-term hormonal or immune-related adaptations. Other variables, including age, smoking habits, place of residence, and number of abortions, did not significantly influence the observed cytogenetic markers.

4. Conclusions

The present study confirms that EC is associated with elevated chromosomal instability in PBLs. Markers such as MNi and NPBs emerged as reliable indicators of this instability, showing significant associations with tumor grade, obesity, and reproductive history. These findings highlight the potential of cytogenetic profiling as a complementary tool in cancer risk assessment and individualized clinical monitoring.

Acknowledgment

This research is funded by the Ministry of Education and Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grants: No. 451-03-136/2025-03/ 200378.

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