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Cytogenetic Study Of Peripheral Blood Leukocytes In Patients With Uterine Mesenchymal Tumors

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ABSTRACT

According to the World Health Organization, uterine tumours rank second in the structure of oncological morbidity in women. Epithelial tumours account for 95% of all genital tumours and only 5% are mesenchymal tumours. Malignant mesenchymal tumours include uterine sarcomas. Annually, 10 cases of uterine sarcomas are diagnosed in 1 million women worldwide. The problems of early diagnosis and screening of malignant uterine mesenchymal tumours (sarcomas) have not yet been solved. Due to the fact that this disease is rare and the treatment of uterine sarcomas remains a pressing problem.

KEYWORDS

Uterine mesenchymal tumours, uterine sarcoma, cytogenetic study.

INTRODUCTION

Objective of the study: To perform a cytogenetic study of peripheral blood leukocytes (examine chromosomal abnormalities) in patients with uterine mesenchymal tumours, to determine a risk group for the development of malignant forms of the disease Uterine sarcomas are rare oncogynaecological neoplasms, accounting for approximately 3-7% of all uterine malignancies. They are mesenchymal tumours, including leiomyosarcoma (LMS), endometrial stromal sarcoma (ES),

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undifferentiated endometrial stromal sarcoma (NDSS) and adenosarcoma (AS). Carcinosarcoma (CSC) or malignant mixed Müller's tumour is now considered a low-differentiated epithelial tumour and will not be included in this review. The incidence of uterine corpus malignant disease per 100,000 in Uzbekistan was 1.6 in 2015 and 1.9 in 2019. The proportion of patients with malignant uterine corpus disease (MVCD) actively detected out of the number of patients with first-time diagnosis in 2015 and 2019 was 33.3%-34.1% respectively. For the year 2019, 640 patients have been registered for EVD, prevalence per 100000 population is 18.2 (2015) and 12.7 (2019). Proportion of patients registered in oncological institutions in 2019 with 5 years or more from the moment of diagnosis at the end of reporting year from the number of registered in 2015 is 49.5%, in 2019 49.5%. 76.6% of patients with stage I-II disease were on the dispensary registry and 14.4% of those with stage 3 disease. The mortality rate of patients per 100000 populations in 2015 and 2019 is 0.6 and 0.8, respectively.

MATERIALS AND METHODS

We studied the results of examination and treatment of 40 patients who were examined for uterine sarcoma in RSNPMC and its Samarkand regional branch during 2011-2019. To establish the diagnosis and determine the extent of tumour spread, the patients underwent a comprehensive examination which included: clinical examination, ultrasound and X-ray examination, computed tomography and magnetic resonance imaging [1-3].

To study the sensitivity, specificity and accuracy of diagnostic techniques (ultrasound CT, MRI) in determining the extent of tumour spread, the results were evaluated by comparing them with the results of intraoperative surgical staging and histological examination of operative material. To study genetic alterations in peripheral blood lymphocytes, cytogenetic studies were performed in 21 patients. The patients' age ranged from 24 to 72 years, mean age was 54.3±5.6 years.

To perform cytogenetic studies we used micromethod of cultivating whole blood lymphocytes of patients with uterine sarcoma by the Arakaki method. Phytohemagglutinin (PHA) was added to activate the mitotic cycle of peripheral blood lymphocytes of patients during cultivation. After 72 hours, when the maximum number of mitoses was reached, colchicine was added to the culture to stop the mitoses at the metaphase stage, the most suitable for analysis [4-6]. The finished preparations were stained with Romanowsky-Giemse stain.

The results showed that the number of metaphases suitable for analysis in the stained preparations was different (Table 1).

Table 1. Values of the test in determining the extent of uterine tumour spread by method of examination

Indicator	Meaning of the test		
	Ultrasonography	Computer tomography	MRI
Sensitivity	74,0	81,5	83,3

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Specificity	**73,3**	**60,0**	**77,0**
Accuracy	**75,0**	**83,3**	**86,4**
Matthewst Correlation Coefficient	**0,43**	**0,756**	**0,812**
Youden index	**0,57**	**0,75**	**0,84**
Area under the ROC curve	**0,549**	**0,683**	**0,663**

The sensitivity of MRI, 83.3%, in diagnosing this criterion, was also almost identical to CT, 81.5%, and ultrasound, 74.0% (P<0.05). Specificity of ultrasound at 73.3% was higher than that of CT at 60% and lower than MRI at 77%, accuracy of MRI at 86.4% was found to be similar to that of CT at 83.3 and higher than that of ultrasound at 75% for this criterion. The Matthewst correlation coefficient between observed and predicted binary classifications returned a value between -1 and +1. The coefficient between 0.43 and 0.812 (mean 0.66) in our case represents the mean value between prediction and observation. This is confirmed by the Youden index, its value ranges from 0 to 1 (inclusive) and it has a non-zero value, in our case the mean is 0.6. The use of the sum of both index values "should normally be recommended", thus the use of ultrasound and/or TVUS in combination with MSCT or MRI in the diagnosis of uterine sarcoma is a probability of making an informed decision [7-11].

The sensitivity, specificity and efficacy of these diagnostic modalities in assessing the local spread of uterine sarcoma were determined for each of these modalities by comparing ultrasound, CT and MRI data with intraoperative surgical staging data and histological examination of surgical material.

In some specimens, mitotic stimulation was zero or so low that a minimal number of metaphases (2 to 8) were used for analysis. There were 5 (23.8%) such patients. In the remaining patients, 16 (76.1%) FGA lymphocyte stimulation was normal. A total of 210 metaphase plates were examined.

Metaphase plates were studied to detect structural changes in chromosomes in peripheral blood lymphocytes and to compare them. The absence of chromosomal aberrations in the studied metaphase plates was detected in 6 patients (Table 2). Chromosome aberrations in peripheral blood lymphocytes were found in 14 (66.7%) out of 21 patients.

Table 2. Number of metaphase plates with aberrations in patients with uterine sarcoma in cytogenetic examination of peripheral blood lymphocytes

Number of metaphase plates with aberrations	Patients with uterine sarcoma (n=21)			P
	abs	M(%)	m	
0 (no aberrations)	6	28,57	9,86	Hee-square = 4,000, p = 0,406
1	6	28,57	9,86	
2	5	23,81	9,29	
4	2	9,52	6,41	
7	2	9,52	6,41	
	21			

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It should be noted that one patient had a lack of FGA stimulation of peripheral blood lymphocytes.

Analysis of the study results according to the number of metaphase plates with aberrations showed that out of 14 patients with uterine sarcoma 6 (42.9%) had metaphase plates with single aberrations and 8 (57.1%) had metaphase plates with 2-7 aberrations.

Patients with uterine sarcoma had a higher number of mutated T lymphocytes in the peripheral blood. Analysis of the results of a study on the number of patients with weak FGA stimulated lymphocytes as one of the possible signs of immunosuppression showed that this sign was observed in 5 (23.9%) out of 21 patients.

Table 3. Analysis of cytogenetic changes in peripheral blood lymphocytes of SM patients

Pathological alterations	Number of patients (40)	17 chromosome pair (17q-),	18 pare (18q-)
Chromosomal abortions as in normal	4 (10%)	normal	Normal
Increased frequency of chromosome abortion with aneuplasia	28 (70%)	deletions of the long arm in 17q-,	Deletions of the long arm in 18q-
Highest incidence of chromosomal structural damage	8 (20%)	the highest incidence of structural chromosomal damage - up to 28%.	the highest incidence of chromosomal structural damage - up to 28%.

This showed that in 10% of cases (4/40) the level of chromosome aberrations did not exceed that in healthy people (the normal rate in healthy people is 2.8%, in the examined patients the figure is up to 3%).

In the remaining cases-70% of patients (28/40) showed an increase in the frequency of chromosome aberrations, these were predominantly deletions of the long arm in the 17th pair (17q-), 18th pair (18q-) and X chromosome as well as the presence of fragments and aneuplications. In 20% (8/40) of patients, the highest incidence of structural chromosomal damage was detected - up to 28% [11-13]. Morphological diagnosis in these patients revealed high mitotic tumor activity, significant cell atypia, hemorrhages, vascular invasion, and, despite the relative monomorphism of the picture, there were some variations in nuclear size, uneven contouring of nuclei in individual cells, coarse fine-grained chromatin, and delicate nucleoli.

Table 4. Informative value of cytogenetic methods in uterine mesenchymal tumours

Histological structure of the tumour	Sensitivity	Specificity	General accuracy
Uterine sarcoma	88%	67%	85%

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Analysis of the results of the study of the number of patients with weak FGA stimulation of lymphocytes showed a predominant frequency of this feature in patients with uterine sarcoma.

Conclusions: Neoplastic transformation is based on stable genetic damage that is transmitted during division and influences the processes of cell proliferation, differentiation and death, which is reflected in the phenotype and biological properties of the tumor.

The results of cytogenetic studies performed on patients with uterine sarcoma showed that chromosomal changes in lymphocytes of peripheral blood are observed more frequently. The study of characteristic genetic changes in lymphocytes in uterine sarcoma, by detecting quantitative and qualitative changes, is a technique for early diagnosis and screening of uterine sarcoma and possibly reveals the cause of immunosuppression.

Thus, the results have shown that patients with uterine sarcoma show marked abnormal chromosomal changes in peripheral blood lymphocytes.

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