sciendo Acta Marisiensis - Seria Medica 2023;69(2):121-127 DOI: 10.2478/amma-2023-0021

RESEARCH ARTICLE

Detection of serum telomerase and fibronectin as precursor markers of cervix cancer in patients with a positive Pap test

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Introduction: Cervical cancer is a type of cancer that appears in cervical cells, in the lower part of the uterus, being characterized by the proliferation of atypical cancerous cells, which can spread rapidly, representing a serious disease, with significant medical and social impact among affected individuals, and a severe evolution particularly when detected in advanced stages. The aim of this study was to evaluate the alterations found in cervical cells, caused by persistent HPV infection, using inflammatory protein biomarkers such as fibronectin and telomerase. **Material and method**: This study included a total of 169 women, both asymptomatic and symptomatic, aged between 30 and 64 years. Those women underwent routine gynecological consultations or were referred to a gynecologist because of their symptoms. After selecting the patients, a Pap test and blood samples (5 ml) were taken. Using a questionnaire, information regarding sexual characteristics and behaviors, as well as personal medical history, were collected. **Results**: The median value for telomerase was 0.1 ng/ml, with a minimum of 0.01 ng/ml and a maximum of 30.09 ng/ml. Based on the telomerase results, 66 (39.1%) patients had positive results (more than 0.215 units) and 103 (60.9%) had negative results. The median value for fibronectin was 3.72 ng/ml with a minimum of 0.55 ng/ml and a maximum of 89.9 ng/ml. Of all women included in the study, 36 (21.3%) had positive results (more than 10 ng/ml), and 133 (78.7%) had negative results. Also, 15.2% of patients with positive telomerase and 16.7% with positive fibronectin presented Atypical Squamous Cells of Undetermined Significance. Mature squamous metaplasia and inflammatory cells have been identified among positive and negative results of fibronectin and telomerase. **Conclusions**: Positive and negative results for fibronectin and telomerase were similar in correlation with cytological results and information about HPV infection or sexual practices/characteristics were similar.

Keywords: cervical cancer, screening, human papillomavirus; fibronectin; telomerase

Received 8 March 2023 / Accepted 11 May 2023

Introduction

Cervical cancer (CC) is a malignant neoplasm that appears in cells from the cervix, the lower part of the uterus that comes in contact with the vagina [1, 2].

It is characterized by the presence of atypical cells in the cervix, that exhibit a capacity for continuous proliferation, leading to a serious disease, with significant medical and social impact among patients and a poor prognostic when detected at advanced stages. Squamous cell carcinoma, which accounts for 70% of cases, is the most prevalent type of CC. Adenocarcinoma is less frequent, comprising approximately 25% of cases, and is more challenging to diagnose due to its location higher in the cervix. Globally, CC is the fourth most common cancer in women, with an estimated 604,000 new cases and 342,000 deaths in 2020. Low- and middle-income countries bear the brunt of this disease, with nearly 90% of new cases and deaths occurring in these regions [3, 4].

In Romania, CC represents a public health problem, due to its late diagnosis, which hinders complete recovery. The main cause and risk factor for CC is infection with Human Papilloma Virus (HPV). This virus is detected in nearly all cases of invasive CC, with one in four women between the ages of 14 and 59 being infected with HPV [5, 6].

HPV is primarily transmitted via direct contact, especially through sexual intercourse [7, 8].

Factors that influence the occurrence of CC are extrinsic factors, such as lifestyle, poor genital hygiene, sexual characteristics (early onset of sexual life and high number of sexual partners), number of births, poor obstetric care, repeated abortions, as well as intrinsic factors, such as age, endocrine or genetic factors [8-10].

Screening for CC using Pap Test proved effective in reducing the incidence of this disease. However, there are certain limitations when it comes to the detection of high-grade cervical intraepithelial neoplasia ([CIN2+]/[CIN3+]) in cytology samples that display atypical squamous cells of undetermined significance (ASCUS) and low-grade intraepithelial lesion (LSIL) [11].

Telomerase is an enzyme that adds repetitive units to the ends of chromosomes. The gradual loss of chromo-

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somes ends limits strictly the growth potential and leads to senescence in normal cells after a defined number of defined divisions. However, tumor cells exhibit an unlimited proliferation potential and become immortal. This is due to the active enzymatic action of telomerase in tumor cells. Therefore, telomerase activation is an important step in carcinogenesis [12, 13]. Telomerase is almost absent in normal cells but expressed at functionally significant levels in the majority (90%) of human cancer cells. By extending telomerase DNA, telomerase is able to counteract the progressive erosion of telomeres that would otherwise occur in its absence. The presence of telomerase activity in cancer cells is correlated with resistance to both senescence and apoptosis [14, 15].

Fibronectin is a glycoprotein composed of a carbohydrate fraction and a protein fraction joined by a covalent bond. Several genetically specified isoforms have been identified for fibronectin. After intracellular synthesis, fibronectins are secreted into the local extracellular matrix where they function. All tissues contain fibronectin [16].

The cellular receptor for fibronectin has been identified in all types of cells, and binding domains to collagen, heparin, fibrin, and actin as well as a cell interaction domain have been identified. Fibronectin has important roles in embryogenesis, in maintaining the integrity of tissues, in hemostasis, and in cancer cell migration [17].

The aim of the study is to evaluate the changes in cervical cells caused by the persistent HPV infection through the protein biomarkers of inflammation: fibronectin, respectively the detection of the telomerase level. Additionally, we investigated the relationships between the extrinsic factors that influence the occurrence of CC and these biomarkers.

Materials and Methods

Target population:

Inclusion criteria: Asymptomatic women or those with symptoms such as abnormal vaginal discharge, genital inflammation or with risk factors such as early onset of sexual activity, high-risk sexual partner, multiple sexual partners, and history of other sexually transmitted infections, immunosuppression, or smoking; aged between 30 and 64. These women presented themselves in public and private gynecological offices for routine gynecological check-ups or due to the appearance of symptoms.

Exclusion criteria: Women under the age of 30 and over 64, with a history of hysterectomy, with invasive surgical procedures in the genital area 12 months before, pregnant, or postpartum women.

The selection of patients was carried out over a period of 18 months (between 2021 and 2022). Eligible women, based on informed consent and inclusion criteria, were provided with information regarding the study protocol, including the steps of the study, and the collection of biological samples (including cervical cytology samples collected using a cervical brush on liquid medium and blood samples to determine telomerase and fibronectin levels). These women were also asked to complete a brief medical questionnaire. Following sample evaluation, they received a phone call to report the results of the tests performed and to inform them about their health status. All data collected and the results obtained were kept confidential. After collection, the cervical cytology samples were refrigerated at 2-8^{o}C, transported, and processed in the laboratory. At the same time, a blood sample of 5 ml was collected from each patient, which was centrifuged at 1200 rpm for 5 minutes, and stored in two Eppendorf tubes.

The collected samples were frozen at -20^{o}C and later used for the determination of serum fibronectin and telomerase levels. Based on a questionnaire, we classified the selected cases according to age, environment, socioeconomic status, education level, marital status, smoking status, alcohol consumption, sexual characteristics (first sexual contact, number of sexual partners, genital infections), personal physiological antecedents (menarche, number of births and abortions) and pathological personal antecedents. Each patient received an informed consent. This questionnaire-based study was approved by The Ethics Committee of the University of Medicine and Pharmacy of Târgu Mureș (no. 530 din 21.11.2019).

Cytopathological technique: Using the Bethesda system, the results of the cytology were classified as follows: Negative for Intraepithelial Lesions or Malignancy (NILM), Atypical Squamous Cells of Undetermined Significance (ASCUS), Atypical Squamous Cells for which a High-grade lesion cannot be excluded (ASCH), Low-grade squamous intraepithelial lesion (LSIL) and High-grade squamous intraepithelial lesion (HSIL) [18, 19]. The microbiological examination of the vaginal discharge included the usage of a growth medium and carrying out an antibiogram, monitoring the possible presence of an infection with various microorganisms: *Trichomonas, Gardnerella, Streptococcus, Staphylococcus, Chlamydia trachomatis, Mycoplasma, Escherichia coli, Klebsiella*, etc.

Telomerase was measured using telomerase repeated amplification protocol (TRAP) assay, which utilized the Telo TAGGG Telomerase PCR enzyme-linked immunosorbent assay (ELISA) kit from Roche Applied Science, Mannheim, Germany. The tests were performed according to the manufacturer's specifications at the Advanced Medical and Pharmaceutical Research Center, within the University of Medicine, Pharmacy, Sciences and Technology "George Emil Palade" of Târgu Mureș, Romania. Samples were considered positive if the absorbance difference was greater than 0.215 units A450nm-A690nm [20].

Serum fibronectin levels were measured using the IBL-International GMBH immune-enzymatic kit, which is based on the ELISA reaction for the quantitative determination of fibronectin. The test utilized the enzyme Streptavidin Horseradish Peroxidase and biotin-conjugated anti-fibronectin antibodies. The standardized working protocol

included in the set of reagents was followed, the recommended reference range for adult patients being 10 ng/ml serum fibronectin, and above this value being a positive result [16].

Statistical analysis

Data was collected in Microsoft Excel spreadsheets. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 23, Chicago, IL, USA). Nominal variables were described as absolutes and relative frequencies (%) and the association between them was analyzed by Pearson's Chi-square test or Fisher's Exact Test. Associations having $P<0.05$ were considered to be significant.

Results

The present paper presents a prospective study that involved 169 patients. The statistical analysis included a varied range of demographic parameters and correlations regarding the values of telomerase and fibronectin in the women surveyed, in connection with sexual characteristics and behaviors.

Correlation of telomerase and fibronectin results with cytological results

The median value of telomerase was 0.10 ng/ml with a minimum of 0.01 ng/ml and a maximum of 30.09 ng/ml. Based on the telomerase results, 66 (39.1%) of the patients had positive results (over 0.215 units) and 103 (60.9%) had negative results. The median value of fibronectin was 3.72 ng/ml with a minimum of 0.55 ng/ml and a maximum of 89.9 ng/ml. Of the total number of patients, 36 (21.3%) had positive results (above 10 ng/ml) and 133 (78.7%) had negative results. 15.2% of telomerase-positive patients and 16.7% of fibronectin-positive patients had atypical squamous cells of undetermined significance (ASC-US). Mature squamous metaplasia and inflammatory cells were similarly identified between positive and negative telomerase and fibronectin results. On the bulletins of the cytology examinations, the colposcopy/biopsy recommendation was indicated, respectively the repetition after 6 months in similar percentages between the positive and negative results of telomerase and fibronectin. Among the microorganisms, Candida albicans and Gardnerella vaginalis germs were highlighted (Table 1).

Correlation of telomerase and fibronectin results with sexual practices/characteristics

In Table 2 we evaluated the influence of sexual practices/characteristics on telomerase and fibronectin results, and the positive and negative results for telomerase and fibronectin were similar in relation to the questions presented in the table. Most of the respondents have a stable sexual partner and in 2/3 of cases, they had at least one sexual act per week. For more than 80% of the respondents, it was not the case of extramarital sexual contact and subsequently requesting a consultation with a specialist doctor, but the itching, abnormal secretions, and bleeding are the elements that would lead patients to see a doctor for a specialist consultation.

Correlation between telomerase and fibronectin results and information regarding HPV infection

The majority of the patients have heard about HPV infection or know the associated diseases caused by HPV infection; however, a significant proportion is mistaken about the modes of transmission of HPV infection (unsterilized needles or blood transfusions/transplants). More than 65% of patients who tested positive for HPV infection perceived it to be a significant health risk. Positive and negative results for telomerase and fibronectin were similar in relation to the questions: *Methods of preventing/reducing HPV infection or knowledge about HPV infection?* as well as *How many times you performed the Pap test.* Regarding the barriers for which patients have not performed the Pap test (cytological), many patients did not consider it necessary (Table 3).

Table 1. Telomerase and fibronectin results in correlation with cytological results

Variables		Result telomerase (ng/ml)		p value	Result fibronectin (ng/ml)		p value
		Pozitive 66 (39.1)	**Negative 103 (60.9)**		**Pozitive 36 (21.3)**	**Negative 133 (78.7)**	
Squamous cells	ASC-US	10 (15.2)	14 (13.6)	0.94	6 (16.7)	18 (13.5)	0.82
	ASC-H	0 (0)	4 (3.9)	0.26	0 (0)	4 (3.0)	0.66
	L-SIL	4 (6.1)	0 (0)	**0.04**	0 (0)	4 (3.0)	0.66
	NILM	10 (15.2)	33 (32.0)	**0.02**	9 (25.0)	34 (25.6)	0.88
	No epithelial cell abnormalities	42 (63.6)	52 (50.5)	0.13	21 (58.3)	73 (54.9)	0.86
Other non-neoplastic changes	Mature squamous metaplasia	23 (34.8)	40 (38.8)	0.71	15 (41.6)	48 (36.1)	0.68
	Inflammatory	26 (39.4)	32 (31.7)	0.39	11 (30.5)	47 (35.3)	0.73
	Atrophy	1 (1.5)	0 (0)	0.72	0 (0)	1 (0.8)	0.51
	No non-neoplastic changes	18 (27.3)	28 (27.2)	0.87	9 (25.0)	37 (27.8)	0.90
Recommendations	Colposcopy/biopsy	4 (6.06)	11 (10.6)	0.46	3 (8.3)	13 (9.7)	0.94
	Repeat after 6 months	13 (19.7)	15 (14.5)	0.49	7 (19.4)	21 (15.7)	0.78
	Repeat after treatment	27 (40.9)	28 (27.2)	0.09	12 (33.3)	43 (32.3)	0.93
	Routine check	22 (33.3)	50 (48.8)	0.06	17 (47.2)	55 (41.4)	0.66
Microorganisms	Candida albicans	8 (12.1)	11 (10.7)	0.97	6 (16.7)	13 (9.8)	0.38
	Gardnerella vaginalis	5 (7.6)	4 (3.9)	0.48	1 (2.8)	8 (6.0)	0.73
	No microorganism identified	53 (80.3)	88 (85.4)	0.51	29 (80.6)	112 (84.2)	0.79

Table 2. Telomerase and fibronectin results in correlation with sexual practices/characteristics

Variables		Result telomerase		p value	Result fibronectin		p value
		Pozitive 66 (39.1)	Negative 103 (60.9)		Pozitive 36 (21.3)	Negative 133 (78.7)	
Marital status	Married	43 (65.1)	71 (68.9)	0.73	22 (61.1)	92 (69.2)	0.35
	Living with partner	3 (4.5)	4 (3.9)	0.83	0 (0)	7 (5.3)	0.14
	Divorced	6 (9.1)	8 (7.76)	0.98	4 (11.1)	10 (7.51)	0.60
	Unmarried	14 (21.2)	20 (19.4)	0.92	10 (27.8)	24 (18)	0.18
First sexual intercourse	Before the age of 18	19 (28.8)	48 (46.6)	**0.03**	12 (33.3)	55 (41.4)	0.37
	After the age of 18	47 (71.2)	55 (53.4)	**0.03**	24 (66.7)	78 (58.6)	0.37
Safe first sexual intercourse	Yes	27 (40.9)	45 (43.7)	0.84	18 (50)	54 (40.6)	0.29
First time living with partner	Before the age of 18	9 (13.6)	18 (17.5)	0.64	5 (13.9)	22 (16.5)	0.81
	After the age of 18	48 (72.7)	74 (71.8)	0.96	26 (72.2)	96 (72.2)	0.86
	NA	9 (13.6)	11 (10.7)	0.74	5 (13.9)	15 (11.3)	0.79
Do you have a stable relationship?	Yes	61(92.4)	95 (92.2)	0.80	33 (91.7)	123 (92.5)	0.91
How many sexual partners did you have until today?	1	23 (34.8)	28 (27.2)	0.37	14 (38.9)	37 (27.8)	0.18
	2	31 (47.0)	57 (55.3)	0.37	13 (36.1)	75 (56.4)	**0.01**
	3	8 (12.1)	14 (13.6)	0.96	6 (16.7)	16 (12.0)	0.52
	4	4 (6.1)	4 (3.9)	0.77	3 (8.3)	5 (3.8)	0.36
Did you have sexual intercourse outside your stable relationship?	Accidentally	6 (9.1)	8 (7.8)	0.98	2 (5.6)	12 (9.0)	0.60
	More than once	2 (3)	2 (1.9)	0.95	1 (2.8)	3 (2.3)	0.76
	From time to time	3 (4.5)	6 (5.8)	0.98	4 (11.1)	5 (3.8)	0.12
	Never	55 (83.3)	87 (84.5)	0.99	29 (80.6)	113 (85.0)	0.59
Duration of sexually active life	Less than 10 years	18 (27.3)	18 (17.5)	0.18	5 (13.9)	31 (23.3)	0.19
	11-20 years	36 (54.5)	55 (53.4)	0.98	21 (58.3)	70 (52.6)	0.57
	More than 21 years	12 (18.2)	30 (29.1)	0.15	10 (27.8)	32 (24.1)	0.72
Frequency of sexual intercourse/ week	1	45 (68.2)	67 (65.0)	0.79	20 (55.6)	92 (69.2)	0.10
	2	17 (25.18)	31 (30.1)	0.60	13 (36.1)	35 (26.3)	0.23
	3	0 (0)	3 (2.9)	0.42	0 (0)	3 (2.3)	0.56
	4	4 (6.1)	2 (1.9)	0.31	3 (8.3)	3 (2.3)	0.15
At what age do you consider appropriate beginning the sexual life?	14-17 years	16 (24.2)	34 (33.0)	0.29	12 (33.3)	38 (28.6)	0.63
	18-21 years	49 (74.2)	66 (64.1)	0.22	24 (66.7)	91 (68.4)	0.97
	22-25 years	1 (1.5)	2 (1.9)	0.68	0 (0)	3 (2.3)	0.56
	Older than 25 years	0 (0)	1 (1.0)	0.84	0 (0)	1 (0.8)	0.68
How much time have passed after sexual intercourse outside your stable relationship and a gynecological consultation?	Less than 2 months	5 (7.6)	5 (4.9)	0.64	1 (2.8)	9 (6.8)	0.43
	2-4 months	0 (0)	1 (1.0)	0.84	0 (0)	1 (0.8)	0.68
	4-6 months	6 (9.1)	7 (6.8)	0.80	2 (5.6)	11 (8.3)	0.72
	NA	55 (83.3)	90 (87.4)	0.60	33 (91.7)	112 (84.2)	0.23
What symptoms made you/ can make you take a gynecological consultation?	Itches	21 (31.8)	31 (30.1)	0.94	10 (27.8)	42 (31.6)	0.72
	Abnormal vaginal discharge	37 (56.1)	50 (48.5)	0.41	17 (47.2)	70 (52.6)	0.59
	Genital lesion	19 (28.8)	25 (24.3)	0.63	6 (16.7)	38 (28.6)	0.11
	Bleeding	28 (42.4)	40 (38.8)	0.76	13 (36.1)	55 (41.4)	0.59

Discussion

The aim of the study was to evaluate the impact of persistent HPV infection on changes in cervical cells, as indicated by protein biomarkers of inflammation, namely fibronectin and telomerase level. We aimed to evaluate the effectiveness of screening in detecting CC precursors. The cohort consisted of 169 women aged between 30 and 64, including asymptomatic women and women with symptoms such as abnormal vaginal discharge, genital inflammation or with risk factors such as early onset of sexual activity, high-risk sexual partners, multiple sexual partners, history of other sexually transmitted infections, immunosuppression or smoking.

By identifying the prevalent circulating HPV genotypes and assessment of the activity levels of telomerase and fibronectin, follow-up strategies and accurate diagnosis of cases can be developed, particularly for infections caused by oncogenic HPV types. We aimed, by correlating the results of fibronectin and telomerase levels, to draw a profile of women who may be predisposed to developing CC.

In order to achieve a detailed correlation between the two markers studied, it was decided to investigate them at the primitive cellular level, starting with the sampling of endocervical cells implemented through the Pap test. In the majority of subjects, 80.5% who present the fibronectin marker within normal parameters, tested negative for an intraepithelial or malignant lesion (NILM), indicating an absence of epithelial cell abnormalities. Similarly, 82.5% of the patients with telomerase within normal limits (below 0.215 units), showed NILM or lack of epithelial cell abnormalities.

In an attempt to identify other factors that could be associated with the two markers given this screening, we tried to demonstrate the predisposition of certain microorganisms for subjects with normal or pathological values. Of the 169 patients included in the study, a majority (141) do not have associated microorganisms. *Candida* and *Gardnerella vaginalis* were taken into account as com-

Table 3. Telomerase and fibronectin results in correlation with HPV infection information

Variables		Result telomerase		p value	Result fibronectin		p value
		Pozitive 66 (39.1)	Negative 103 (60.9)		Pozitive 36 (21.3)	Negative 133 (78.7)	
Did you know about HPV infection?	Yes	60 (90.9)	88 (85.4)	0.41	29 (80.6)	119 (89.5)	0.24
How can you acquire an HPV infection?	Heterosexual intercourse	15 (22.7)	19 (18.4)	0.62	8 (22.2)	26 (19.5)	0.90
	Homosexual intercourse	26 (39.4)	41 (39.8)	0.91	13 (36.1)	54 (40.6)	0.76
	Intimate touch	39 (59.1)	61 (59.2)	0.88	18 (50.0)	82 (61.7)	0.28
	Unsterile needles	41 (62.1)	61 (59.2)	0.82	19 (52.8)	83 (62.4)	0.39
	Blood transfusion/organ transplantation	48 (72.7)	75 (72.8)	0.87	24 (66.7)	99 (74.4)	0.47
Do you consider that HPV is a risk for your health status?	High risk	46 (69.7)	72 (69.9)	0.88	24 (66.7)	94 (70.7)	0.79
	Medium risk	14 (21.2)	14 (13.6)	0.27	5 (13.9)	23 (17.3)	0.81
	Low risk	2 (3.0)	0	0.30	2 (3.0)	1 (1.0)	0.91
	I don't know	4 (6.1)	16 (15.5)	0.11	5 (13.9)	15 (11.3)	0.89
Which are the diseases caused by HPV?	Cervical cancer	2 (3.0)	3 (2.9)	0.66	2 (5.6)	3 (2.3)	0.63
	Anal cancer	2 (3.0)	3 (2.9)	0.66	2 (5.6)	3 (2.3)	0.63
	Oral cancer	11 (16.7)	11 (10.7)	0.37	4 (11.1)	18 (13.5)	0.91
	Genital condylomatosis	2 (3.0)	3 (2.9)	0.66	2 (5.6)	3 (2.3)	0.63
Choose the methods for prevention/reduction of HPV infection	One sexual partner	15 (22.7)	22 (21.4)	0.99	8 (22.2)	29 (21.8)	0.86
	Using the condom	16 (24.2)	21 (20.4)	0.69	9 (25.0)	28 (21.1)	0.78
	Vaccination	12 (18.2)	16 (15.5)	0.80	6 (16.7)	22 (16.5)	0.82
	Personal hygiene, soap and water after sexual intercourse	4 (6.1)	6 (5.8)	0.79	1 (2.8)	9 (6.8)	0.61
	Pap test	11 (16.7)	14 (13.6)	0.74	7 (19.4)	18 (13.5)	0.53
Knowledge about HPV	Most of the HPV infection need medical treatment	24 (36.4)	34 (33.0)	0.77	12 (33.3)	46 (34.6)	0.37
	Cervical cancer or dysplasia treatment eradicates the infection	13 (19.7)	19 (18.4)	0.97	6 (16.7)	26 (19.5)	0.74
	Genital condylomatosis is caused by the same HPV types that causes cervical cancer	25 (37.9)	22 (21.4)	**0.023**	10 (27.8)	37 (27.8)	0.15
	Most of the cases of cervical cancer are caused by HPV infection	42 (63.6)	49 (47.6)	0.10	18 (50.0)	73 (54.9)	0.79
	Women with HPV infection cannot receive the vaccine	17 (25.8)	23 (22.3)	0.75	8 (22.2)	32 (24.1)	0.93
	Did you receive a vaccine against HPV infection?	7 (10.6)	2 (1.9)	**0.009**	2 (5.6)	7 (5.3)	0.35
How many times did you undertake a Pap test?	Never	6 (9.1)	8 (7.8)	0.98	3 (8.3)	11 (8.3)	0.73
	2-3 times	19 (28.8)	26 (25.2)	0.73	11 (30.6)	34 (25.6)	0.69
	More than 3 times	41 (62.1)	69 (67.0)	0.62	22 (61.1)	88 (66.2)	0.71
What made you not to go for a Pap test?	Time	8 (12.1)	27 (26.2)	**0.04**	4 (11.1)	31 (23.3)	0.17
	Money	3 (4.5)	8 (7.8)	0.59	2 (5.6)	9 (6.8)	0.90
	Fear of a positive result	9 (13.6)	14 (13.6)	0.81	6 (16.7)	17 (12.8)	0.74
	I considered that is not necessary	46 (69.7)	54 (52.4)	**0.03**	24 (66.7)	76 (57.1)	0.39

mon microorganisms. If out of the 169 patients, 19 have *Candida*, the number of those cases where *Gardnerella* was identified is slightly reduced, with only 9 cases. Among those who are positive for *Candida*, compared to the total number of people with abnormal fibronectin, the percentage of 16.7% exceeds that of women with fibronectin in the normal range (9.8 %). The percentage values indicate that there may be a link between these microorganisms and the abnormal presence of fibronectin, but the relationship is not statistically significant (*p-0.38*). In addition, among those found positive with *Gardnerella vaginalis*, the higher percentage is in favor of those with normal fibronectin, representing a percentage of 6.0%. In the case of pathological telomerase, the discussions are maintained as in the case of fibronectin, identifying the same link between values above 0.215 units and microorganisms.

In the gynecological area, especially related to the suspicion of CC, other non-neoplastic changes have also been studied. Thus, out of 169 subjects, a number of 46 (27.2%) showed no non-neoplastic changes, while 23 (34.8%) of those with pathological values of telomerase, showed mature squamous metaplasia, and 26 (39.4%) from samples showed inflammatory changes. From Table 1, regarding the relationship between fibronectin and the presence of other non-neoplastic changes, the percentage values for mature squamous metaplasia, respectively for inflammatory changes are similar both in the case of the subgroup with normal fibronectin and in the subgroup with abnormal fibronectin (p>0.05). It appears that the patients studied in the sample are not influenced by the presence of other non-neoplastic changes.

During the study, according to Table 2 and a questionnaire applied to each patient, we noticed that married patients have a higher percentage and that the results for both fibronectin and telomerase were similar between positive and negative patients.

The questionnaire also included questions that were meant to highlight the type of sexual contact practiced, family, and extramarital relationships or cohabitation. However, there were no statistically significant changes in fibronectin and telomerase values.

In an attempt to lay the foundations for screening as complex as possible for the type of cancer studied, the questionnaire involves the doctor-patient relationship, in

order to establish a broad basis of support regarding the symptoms and signs that precede the reason for possible medical consultation.

Of the total number of patients, a significantly large number, namely 145 (85.8%), consider that they do not require specialist control in the gynecological, urological, or dermatological area.

Including other symptoms and signs that direct the patient to a consultation, such as vaginal itching, bleeding after sexual intercourse, the presence of irregular periods, disorders of sexual dynamics, and the presence of genitourinary infections, in patients who have neglected themselves from a medical point of view, showed changes in fibronectin and telomerase but not in a statistically significant way.

HPV infection, being the main cause of CC, it was necessary to evaluate the knowledge of participants regarding this aspect, thus, we found that both the people with abnormal studied markers and those with normal intervals are not aware of the influence of this papillomavirus.

In addition to the other possible clinical manifestations mentioned above, the clinical picture of HPV infection can generate the appearance of genital condylomatosis, a phenomenon that must represent a decisive moment for women to undergo a specialist consultation. However, our patients do not seem to be aware of this phenomenon, so a majority do not associate the two conditions.

In Table 3, regarding the frequency of performing the Pap test, we have shown similar results of the two markers, with no statistically significant differences between the percentages related to their normal and abnormal values. A worrying aspect is that time or the fear of an abnormal result, are barriers for which many women have not performed the Babes Pap test until now, despite the fact that it is the first non-invasive test for performing the appropriate screening and for prevention. In addition, for adequate information, all physicians caring for patients with CC predisposition or presenting with specific signs and symptoms should notify their patients of this screening according to individual specifications.

Conclusion

By corroborating the results with inflammatory proteins, fibronectin, and telomerase levels, we wanted to draw the profile of women prone to CC, respectively to create an algorithm for the management of patients with intraepithelial lesions, by the correct and fast establishment of a diagnosis, which to allow adequate treatment of dysplasia.

Whereas increased telomerase activity has been demonstrated in many human cancers, and fibronectin is an intensively studied protein in carcinogenesis, in our study, positive and negative results for these markers in correlation with cytological results, respectively concerning information about HPV infection or sexual practices/characteristics were similar. We did not identify an increase in abnormal values of these markers in women with risk factors (early onset of sexual activity, high-risk sexual partner, multiple sexual partners, history of other sexually transmitted infections).

Author Contributions

VS designed the study. prepared the material. statistically processed and analyzed the data. interpreted the results. developed and edited the manuscript. CU coordinated and monitored the study activities and critically reviewed the manuscript. SGT analyzed the data and critically revised the manuscript. RFF and ZK reviewed the material used for the study and critically reviewed the manuscript. The BMA helped to collect and enter the questionnaires into the study database. All authors read and approved the final manuscript.

Acknowledgments

This work was supported by the University of Medicine, Pharmacy, Sciences and Technology of Târgu Mureș Research Grant number 615/14/17.01.2019.

Conflicts of Interest

The authors declare no conflict of interest.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

References

1. Huang J., Qian Z., Gong Y., Wang Y., Guan Y., Han Y., et al. Comprehensive genomic variation profiling of cervical intraepithelial neoplasia and cervical cancer identifies potential targets for cervical cancer early warning. J. Med. Genet. 2018; 56:186–194. doi: 10.1136/jmedgenet-2018-105745.
2. American Cancer Society. Cancer facts & figures 2018. Atlanta, GA: American Cancer Society, 2018.
3. World health Organization (2020). Global strategy to accelerate the elimination of cervical cancer as a public health problem. https:// www. who. int/ publi catio ns/i/ item/ 97892 40014 107
4. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021:71:209–49. doi:10.3322/caac.21660.
5. Todor RD, Bratucu G, Moga MA, Candrea AN, Marceanu LG, Anastasiu CV. Challenges in the Prevention of Cervical Cancer in Romania. Int J Environ Res Public Health. 2021 Feb 10;18(4):1721. doi: 10.3390/ijerph18041721.
6. Voidăzan, T.S.; Budianu, M.A.; Rozsnyai, F.F.; Kovacs, Z.; Uzun, C.C.; Neagu, N. Assessing the Level of Knowledge, Beliefs and Acceptance of HPV Vaccine: A Cross-Sectional Study in Romania. Int. J. Environ. Res. Public Health 2022, 19, 6939. https://doi.org/10.3390/ijerph19116939
7. Chatterjee S., Gupta D., Caputo T.A., Holcomb K. Disparities in Gynecological Malignancies. Front. Oncol. 2016; 6:36. doi: 10.3389/fonc.2016.00036.
8. Bosch FX, De Sanjosé S. The epidemiology of human papillomavirus infection and cervical cancer. Dis Markers 2007; 23(4): 213–227.
9. Kahn J.A. HPV vaccination for the prevention of cervical intraepithelial neoplasia. N. Engl. J. Med. 2009; 361:271–278. doi: 10.1056/NEJMct0806938.

10. Tekalegn Y, Sahiledengle B, Woldeyohannes D, Atlaw D, Degno S, Desta F, et al. High parity is associated with increased risk of cervical cancer: Systematic review and meta-analysis of case-control studies. Womens Health (Lond). 2022;18:17455065221075904. doi: 10.1177/17455065221075904.
11. Chrysostomou A.C, Stylianou D.C, Constantinidou A, Kostrikis L.G. Cervical Cancer Screening Programs in Europe: The Transition Towards HPV Vaccination and Population-Based HPV Testing. Viruses. 2018 Dec 19;10(12):729. doi: 10.3390/v10120729.
12. Panczyszyn A., Bernacka E.B., Głab G., Telomeres and Telomerase During Human Papillomavirus Induced Carcinogenesis, Mol Diagn Ther, 2018 22:421–430 https://doi.org/10.1007/s40291-018-0336-
13. Molano M., Martín D.C., Acosta P.M, Hernández G, Cornall A., Buitrago O., et al. Telomerase activity in cervical scrapes of women with high grade cervical disease: A nested case control study, Oncology Letters. 2017; 15:354-360, DOI: 10.3892/ol.2017.7324
14. He, H.; Pan, Q.; Pan, J.; Chen, Y.; Cao, L. Study on the correlation between hTREC and HPV load and cervical CINI/II/III lesions and cervical cancer. J. Clin. Lab. Anal. 2020; 34, e23257. https://doi.org/10.1002/jcla.23257.
15. Ji, W.; Lou, W.; Hong, Z.; Qiu, L.; Di, W. Genomic amplification of HPV, h-TERC and c-MYC in liquid-based cytological specimens for screening of cervical intraepithelial neoplasia and cancer. Oncol. Lett. 2019; 17:2099–2106. https://doi.org/10.3892/ol.2018.9825.
16. https://resources.rndsystems.com/pdfs/datasheets/dfbn10.pdf
17. Voidăzan, S.T.; Dianzani, C.; Husariu, M.A.; Geréd, B.; Turdean, S.G.; Uzun, C.C.; Kovacs, Z.; Rozsnyai, F.F.; Neagu, N. The Role of p16/ Ki-67 Immunostaining, hTERC Amplification and Fibronectin in Predicting Cervical Cancer Progression: A Systematic Review. Biology 2022, 11, 956. https://doi.org/ 10.3390/biology11070956
18. Nayar R, Wilbur DC. The Pap test and Bethesda. Cancer Cytopathol 2015;123:271–81.
19. Jahic M, Jahic E. Diagnostic approach to patients with atypical squamous cells of undetermined significance cytologic findings on cervix. Med Arch 2016;70:296–8.
20. https://www.lsbio.com/elisakits/manualpdf/ls-f12713.pdf