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State of HPV16 integration in Lithuanian women with cervical neoplasia

Research Article

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Abstract: Cervical cancer morbidity and mortality in Lithuania is one of the biggest in the European Union. The main risk factor of cervical cancer is human papillomavirus (HPV). The deletion of the HPV E2 gene influences HPV DNA integration into the cell genome, as well as a rapid progression of cervical lesions. The purpose of this study is to determine HPV, its types, and HPV 16 integration in different grades of cervical intraepithelial neoplasias (CIN). 253 women with cytological lesions were involved in the study. After a histology, 31 women were diagnosed with CIN I, 35 with CIN II, and 51 with CIN III. The biggest prevalence of HPV infection was detected in women younger than 25 years old (69.7%) and in women with CIN II (90.9%). HPV 16 was detected in 67.8% of all cases, with the highest prevalence in CIN III (84.4%). A partial integration form was detected in 65.0% of HPV 16 infected women, a complete virus integration in 26.5%, and an episomal form in 8.4% of cases. Our study concludes that in all the cases confirmed using a histology, the partial virus integration form of CIN was identified the most. It was less frequently detected in CIN I cases (60.0%), but more frequently in CIN II and CIN III cases (72.8 and 69.3%, respectively).

Keywords: *HPV prevalence • HPV 16 integration • Cervical intraepithelial neoplasia*

1. Introduction

The current survey indicates that every year, around the world, about 500 thousand women are diagnosed with cervical cancer and 270 thousand die from the disease. The average cervical cancer morbidity rate is 11.9 cases/100,000 women in European countries [1], whereas in Lithuania, it reached 32.1 cases/100,000 women in 2004. Annually, in Lithuania, 500 new cases of cervical cancer are diagnosed and about 250 women die from the disease [2]. Morbidity and mortality caused by cervical cancer in Lithuania were growing during the past decades, and even exceeded the average in European countries. There have been no tendencies towards reduction so far. Cervical cancer in Lithuania is the 5th most frequent form of cancer among women, and among women ages 15-44 years-old, it is the second most frequent one. Nowadays, more and more invasive cervical cancer cases are determined in women younger than 30 years of age [3].

There is no doubt that human papillomavirus (HPV) is the main risk factor in the carcinogenesis of the cervix. The estimates of the HPV prevalence in Lithuania are not accurate. However, in Northern Europe, the region in which Lithuania belongs to, about 8.0 percent of women in the general population are infected with cervical HPV infection at a given time [1]. The two

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most commonly observed high-risk HPV types found in cervical cancer are HPV 16 and 18. These types of viruses are determined in approximately 26 percent of healthy women and nearly 100 percent of women with the diagnosis of invasive cervical cancer. From 30 to 60 percent of women with different cervical intraepithelial neoplasias (CIN) are infected with these types depending on the grade of CIN [4]. In Lithuania, 92.0 percent of invasive cervical cancer is attributed to HPV 16 or 18 [5]. Despite the fact that HPV infection and oncogenic HPV types are the main risk factors of developing cervical cancer, the important roles in cancerogenesis are also played by the viral load in cells, persistent HPV infection, infections with multiple oncogenic HPV types, and the state of the immune system. Therefore, recently, more attention has been focused on molecular markers, such as virus activity markers (HPV mRNR), gene expression (for instance, *p16*), and DNA methylation changes [6,7]. The deletion of the HPV E2 gene plays many important roles, such as the integration of HPV DNA into a host cell genome and, referring to other researchers [8,9], provides the possibility of a more rapid progression of cervical intraepithelial lesions from low to high and faster progression to invasive cervical cancer.

During the integration process of HPV DNA into the host cell DNA, the viral genome usually breaks in the E1/E2 region. This break usually leads to the loss of the E1 and E2 regions. The deletion of the E2 gene, which encodes proteins that suppress the transcriptional control of the E6 and E7 regions, results in the uncontrolled expression of the E6 and E7 oncoproteins. The binding of E7 to the retinoblastoma gene product (pRb) leads to the transcriptional deregulation of cell-cycle control and results in uncontrolled rapid cell proliferation. The binding of E6 inactivates and degrades p53. This results in the loss of p53 induced cell apoptosis, which thus increases chromosomal instability, cell transformation, mutation, and tumor formation [10,11]. It seems that HPV integration into the host genome is related to the progression of the alteration of cervical intraepithelial cells from the polyclonal to the monoclonal status in the CIN, which plays a fundamental role in the progression from low-grade to high-grade cervical neoplasia. Virus integration is determined in about 90 percent of cervical cancer cases and is more common in high-risk HPV than in low-risk HPV. The deletion of some genes is usually determined during the virus integration process into host cell genome. Despite the fact that the deletion of various parts of the virus genome is detected in virus integrated transcripts, constant deletion of some certain genes is also found in the transcripts. On numerous occasions, E2 gene deletion can be identified [9].

The aim of our study is to identify the prevalence of HPV infection, different HPV types, and HPV 16 integration status into cervical epithelial cells according to E2 gene deletion in Lithuanian women with different grades of cervical intraepithelial neoplasia.

2. Material and Methods

2.1. Study population

Two hundred fifty-three women, after performing a *Pap* smear through a screening program and attending the Vilnius University Hospital Santariskiu Clinic with diagnoses of various cervical intraepithelial lesions, were involved in our study. A hundred ten women had a cytological diagnosis of ASCUS (atypical squamous cells of undetermined significance), 6 had ASC–H (atypical squamous cells – not possible to exclude HSIL), 2 had AGC (atypical glandular cells), 44 had LSIL (low grade squamous intraepithelial lesion), and 91 had HSIL (high grade squamous intraepithelial lesion). The study period was from March 1st of 2008 to July 31st of 2008. All the women signed the Invitation and Informed Consent Form. Study Protocol and the Invitation and Informed Consent forms were confirmed by the Lithuanian Bioethics Committee (permission to perform biomedical research was received on February 29th of 2008 Nr.15.). Pregnant women were not included in the study. A biopsy was taken from patients who required a histological test according to the women supervision algorithm. Afterwards, the histological examination cervical intraepithelial neoplasia was confirmed for 117 women: 31 had CIN I, 35 had CIN II, and 51 had CIN III. For 114 women, dysplasia was not confirmed by histology and 5 women were diagnosed with cancer. For 17 women, the biopsy was not performed according algorithms. Women with CIN II, CIN III, or invasive cervical cancer were referred to a hospital for further treatment. Those with CIN I were subjected to further observation. The HPV infection, different HPV types, and HPV 16 E2 gene deletion were detected in all the women through polymerase chain reaction (PCR) in the Institute of Oncology, Vilnius University.

2.2. HPV, specific HPV types, and HPV 16 E2 gene deletion detection by PCR

The material used for the determination of HPV infection (cells taken from the cervical epithelium) was taken using a sterile cervical brush and was transferred into 1 ml of PBS buffer solution. DNA was isolated and HPV tests were performed using the fresh (not frozen) material. The DNA was isolated using a commercial *Sorpoclean*

DNA isolation Kit (Joint-Stock Company "SORPO Diagnostics," Lithuania) according to the manufacturer's recommendations and was stored at -20ºC for future examination.

2.2.1. Characteristics of PCR procedure for HPV and its identifications of types

In order to determine the HPV, a PCR reaction was performed using a 50 µl of mixture, which consisted of 45 µl of commercial *HPV Master Mix* (Joint-Stock Company "SORPO Diagnostics," Lithuania) and 5 µl of investigated DNA, following the manufacturer's recommendations. Prior to performing PCR, which was necessary to determine the HPV, the presence of the β globine gene was checked for in all the DNA samples. Each PCR for HPV determining was performed by using a positive control, which was included in the commercial kit. For a negative control, the samples without DNA (with deionized water) were used. All the HPV positive samples were investigated further: several PCRs were performed to identify different virus types, and for this purpose, commercial *HPV 16, 18 Master Mix*, *HPV 31, 33, 59 Master Mix*, and *HPV 6, 11, 45 Master Mix* (Joint-Stock Company "SORPO diagnostics," Lithuania) were used.

2.2.2. Detection of HPV 16 E2 gene deletion

For the detection of HPV 16 E2 gene deletion, PCR was performed using specific primers in order to determine 16 HPV E2 gene fragments. Three E2 gene fragments (475bp, 477bp, and 276bp) were amplified. To perform reactions, a *REDTaq ReadyMix* (Sigma, USA) PCR kit was used and was performed according to the protocol. The PCR reaction cycle consisted of the primary denaturation cycle at 95°C, which takes 4 minutes, followed by 40 cycles, each consisting of the denaturation at 95°C (1 minute), the annealing of primers at 58°C (2 minutes), and the extension of primers at 72°C (1 minute 30 seconds). At the end, 1 final primer's extension cycle was performed at 72°C, which takes 7 minutes [12].

2.2.3. Visualization of PCR products by electrophoresis

Amplificated PCR products were analyzed using electrophoresis. Electrophoresis was performed using 2 percent agarose gel stained with ethidium bromide. After electrophoresis, ethidium bromide stained products were analyzed using a UV-light (320 nm) transilluminator. The results were pictured and saved on the computer.

2.3. Statistical analysis

All women were analyzed according to age groups, diagnosed cervical intraepithelial neoplasia (histological diagnosis), HPV infection, and HPV 16 E2 gene integration status. All studied features were qualitative; therefore, the data was analyzed in tabular frequency forms. Category frequencies of separate features (n) and their respective proportions (%) were submitted in these forms. In order to check the hypothesis about the independence of two qualitative features χ^2, and if the number of observations was small, an exact Fisher's test was used. For evaluation of the linear dependence of graded qualitative features, the Mantel-Haenzel χ^2 test was used. For checking the hypotheses, a significance level $p<0.05$ was chosen. Study data analysis was performed by using SPSS 13 software packages.

3. Results

Two hundred fifty-three women with different intraepithelial lesions according to the *Pap* smear (from ASCUS to HSIL), and who attend the Vilnius University Hospital Santariskiu Clinic, were involved in the study from March 1st of 2008 to July 31st of 2008. Our study included women whom already acquired cervical intraepithelial lesions, which were determined by a *Pap* smear in different primary outpatient clinics. All women were divided into 5 groups according to their ages: <25, 26-35, 36-45, 46-55, and >56 years. The average age of the women was 37.9 (from 18 to 70 years, SD+/-10.53). The biggest group of women was in 25-36 year old division (74 women). Cytological changes were distributed as follows: 110 had ASCUS, 6 had ASC–H, 2 had AGC, 44 had LSIL, and 91 had HSIL. Most of the women had ASCUS lesions. The fresh cervical cells were taken for HPV testing, typing, and investigation of HPV16 E2 gene deletion. The colposcopy was performed on all women and if the changes were detected, the biopsy was taken. For 17 women, colposcopy changes were not detected and only inflammatory or atrophic changes were stated. The biopsy results were as following: 114 women had no detected histological changes, 31 had grade I cervical intraepithelial neoplasia (CIN), 35 had CIN II, 51 had CIN III, and 5 were diagnosed with invasive cervical cancer.

3.1. HPV prevalence in study population

All women were screened with a HPV test. After detecting HPV by PCR, it was estimated that 46.3 percent of all women (117 out of 253 cases) had HPV infection. Statistically significant differences of HPV infection were detected while analyzing women by separate age groups ($p=0.0002$). HPV infection was determined mostly in women with cervical intraepithelial lesions (by cytology) younger than 25 years of age.

Table 1. HPV prevalence by histological diagnosis.

Histological diagnosis	HPV prevalence				All cases
	HPV-negative		HPV-positive		
	n	%	n	%	n
Dysplasia not confirmed by histology	85	74,6	29	25,4	114
CIN I	13	41,9	18	58,1	31
CIN II	4	11,4	31	88,6	35
CIN III	18	35,3	33	64,7	51
Invasive cancer	3	60,0	2	40,0	5
Histology not performed	13	76,5	4	23,5	17
Total	136	53,7	117	46,3	253

For χ^2 test $p < 0,0001$, for Mantel-Haenszel χ^2 test $p < 0,0001$

Table 2. Prevalence of HPV-16 and other HPV types by histology.

Histological diagnosis	Virus types				All cases
	HPV-16		Other HPV		
	n	%	n	%	n
Dysplasia not confirmed by histology	19	65,5	10	34,5	29
CIN I	10	62,5	6	37,5	16
CIN II	23	74,2	8	25,8	31
CIN III	27	84,4	5	15,6	32
Invasive cancer	2	100,0	0	0,0	2
Hystology not performed	2	50,0	2	50,0	4
Total	83	72,8	31	27,2	114

For χ^2 test $p = 0,221$, for Mantel-Haenszel χ^2 test $p = 0,06$

In this group, HPV infection was identified in 69.7 percent of cases (23 out of 33 women). The infection was rather frequent in the age group of 26-35 year olds. In this group, HPV infection reached 57.0% (45 out of 79 women). In the groups between ages 36-45 and ages 46-55, HPV infection was less common and reached 42.7 and 25.4 percent, respectively. There were only 10 women older than 56 in the last group and HPV infection was detected in 4 of them. Consequently, HPV infection was more frequently identified in women younger than 25 years of age (χ^2 test p=0.0002, Mantel-Haenszel χ^2 test p<0,0001). According to cytology, the majority of HPV infection cases was determined in women with HSIL (63.0 percent), while less cases were associated with LSIL (47.7 percent) and ASCUS (34.6 percent). The study revealed statistically significant differences between HPV infections through cytological findings: prevalence of HPV infection is less common in lower grade cervical intraepithelial lesions (ASCUS and LSIL), in comparison to prevalence of HPV infection in higher grade cervical intraepithelial lesions (HSIL) (χ^2 test p = 0,0006, Mantel-Haenszel χ^2 test p = 0,0002).

3.2. HPV prevalence among women according to cervical biopsy material

Cervical pathology by histology was confirmed in 51.7 percent of the women (122 cases from 236 biopsied women). After HPV testing among the women with no cervical changes in biopsy material, HPV infection was detected in 25.4 percent of women (29 out of 114 cases), in 58.1 percent of women with CIN I (18 cases per 31), and in 88.6 percent of women with CIN II (31 out of 35 cases). Among the women with CIN III, HPV infection was found to be 64.7 percent (33 out of 51 cases). In women with high grade cervical dysplasia (CIN II or III), HPV was determined more frequently than in women with low grade (CIN I) (p<0,0001) (Table 1). It should be noted that 5 women were diagnosed with cervical cancer by biopsy, and 2 these 5 women were infected with HPV.

3.3. HPV types prevalence

Following HPV typing, among 117 HPV-positive women, HPV 16 was identified in 68.4 percent of women (80 out of 117 cases). HPV 18 was identified in only 5.1 percent (6 out of 117 cases). In single cases, HPV 31 and double

Table 3. Data of HPV16 E2 gene deletion in study population.

HPV-16 type integration state	n	%
Complete virus integration	22	26,5
Partial (mixed) integration (2 fragments deletion)	45	54,2
Partial (mixed) integration (1 fragment deletion)	9	10,8
Episomal virus form	7	8,4
Total	83	100,0

infection with HPVs 16 and 18, HPVs 16 and 31, HPVs 16 and 59, and also HPVs 31 and 33 were detected (only one case in each); HPVs 31, 59, and 45 were identified in 2 cases in each, while HPV 33 was identified in 4 cases each. In all age groups, the prevalence of HPV type 16 was the same and about 70 percent of all women in different age groups were infected with this virus type (p=0,027). Similar frequencies of HPV 16 were identified in all cytological categories (for χ^2 test p = 0.0005, and for Mantel-Haenszel χ^2 test p = 0.0001).

3.3.1. Prevalence of HPV 16 by histology

In women with CIN I, HPV 16 was identified in 62.5 percent of women (10 women out of 16 HPV were infected), in CIN II, 74.2 percent (23 women out of 31 HPV were infected), and in CIN III. 84.4 percent (27 women out of 32 HPV were infected). This virus type was detected more rarely in CIN I and in cases when histology did not confirm the results of *Pap* smear and dysphasia was not detected (Table 2). However, we have not stated the statistically significant differences of HPV 16 prevalence in the different grades of CIN, which are due possibly to the insufficient number of cases in different groups.

3.4. HPV 16 E2 gene deletion analysis

All 83 HPV 16 positive women were screened for HPV 16 E2 gene deletion. There were three groups according to E2 gene integration statuses: completely integrated virus form (when none of the three E2 gene fragments were not amplified), partial (mixed) virus integration (when one or two E2 gene fragments were amplified), and non-integrated, or episomal, virus form (when all three E2 gene fragments were amplified). Exploring HPV 16 E2 gene separate fragments, it was found that the partial (mixed) virus integration form was detected in the majority of the cases with 65.0 percent of HPV 16 infected women (54 cases out of 83). Complete virus integration was detected in 26.5 percent of cases (22 women out of 83), while the episomal form of HPV 16 was found in only 8.4 percent of cases (7 women out of 83) (Table 3).

3.4.1. HPV 16 E2 gene deletion analysis by histology

Eighty out of 83 HPV 16 infected women followed up with a biopsy and for 60 of them, cytological changes were confirmed through histology. In all cases of the approved pathology (CIN I, CIN II, and CIN III), mostly the partial (mixed) virus integration form was identified. It was less frequently detected in CIN I cases (60.0 percent), but more frequently in CIN II and CIN III cases

Table 4. Distribution of HPV16 E2 gene deletion in women by histology (n=83*).

Histology	Type of integration	n	%
No histology (n=3)	Complete virus integration	3*	-
No dysplasia (n=20)	Complete virus integration	8	40,0
	Partial (mixed) integration (2 fragments deletion)	11	55,0
	Episomal virus form	1	5,0
CIN I (n=10)	Complete virus integration	3	30,0
	Partial (mixed) integration (2 fragments deletion)	6	60,0
	Episomal virus form	1	10,0
CIN II (n=22)	Complete virus integration	6	27,3
	Partial (mixed) integration (2 fragments deletion)	12	54,6
	Partial (mixed) integration (1 fragments deletion)	4	18,2
	Total	22	100
CIN III (n=26)	Complete virus integration	3	11,5
	Partial (mixed) integration (2 fragments deletion)	14	50,1
	Partial (mixed) integration (1 fragments deletion)	5	19,2
	Episomal virus form	5	19,2
SCC (n=2)	Partial (mixed) integration (2 fragments deletion)	2	-

Fisher's exact test p=0,2521
** 3 patients not tested for histology.*

(72.8 percent and 69.3 percent, respectively). However, differences were not statistically significant (Table 4). It should be noted that in 40 percent of cases, when cytological changes were not confirmed by histology, complete virus integrations were detected. HPV 16 episomal forms in sporadic cases were detected only in CIN I and CIN III patients, and in women with no confirmed pathology.

4. Discussion

The assumption that HPV infection can lead to the formation of cervical cancer was made in 1970. It provided an impetus to explore HPV and the mechanism of cervical cancer development in detail. In 2008, the Nobel Prize for achievements in medicine was given to Harold Zur Hausen, who separated HPV from cervical cancer biopsic specimens and detected the causality between HPV and cervical cancer in 1983. In Lithuania, studies clarifying HPV function in cervical carcinogenesis were started in 1999. M. Kliucinskas and his contributors analyzed factors affecting HPV prevalence and survival and their relationship with cervical intraepithelial lesions [13,14]. It was stated that infection with high-risk HPV in the analyzed women reached 25.1 percent and that urban women were more frequently infected with HPV in comparison to rural women (27.0 vs. 11.1 percent, $p<0.05$). In the Institute of Oncology, Vilnius University, HPV and the influence of HPV types and variants on the risk of cervical cancer were investigated [5,15]. HPV infection in Lithuanian women with cervical cancer was identified to be at 92.0 percent, whereas in healthy women, HPV infection was found to be at 23.6 percent ($p<0.0001$). This study was aimed in order to identify the individual virus types, the most frequent type being HPV 16. Lithuanian studies showed a significantly higher prevalence of HPV, compared to the average HPV prevalence in Europe and worldwide [3,11]. Thus, morbidity and mortality due to cervical cancer in Lithuania are higher compared with the European average.

As the study of HPV prevalence in different cervical lesions suggests, prevalence depends on the level of different cervical intraepithelial lesions. Researchers report that HPV is found in over 50 percent of those with ASCUS, and in more than 80 percent of those with HSIL, especially if HSIL is confirmed by histology as CIN II or CIN III. The studies indicate that women with different cervical intraepithelial lesions, as well as women with no cytological changes in the cervix, show a decreasing prevalence among the age group of 35, and the second virus prevalence increases at the age of 50 [16,17]. Our data showed that 46.3 percent of women with different levels of cervical intraepithelial lesions (from ASCUS to HSIL) were infected with HPV. The highest HPV prevalence was detected among young, 25 years old women (69.7 percent). According to a histological diagnosis, more frequent HPV infections were detected in CIN II patients (88.6 percent) than in CIN I patients (58.1 percent) or in CIN III patients (64.7 percent) ($p<0.0001$). Nevertheless, it is known that a HPV infection can clear up by itself. However, other research data suggests that changes in the cells may remain even up to 6 months, though the virus can be eliminated from the body before that time [18]. This apparently could have had an impact on our results because HPV prevalence in CIN III was less than in CIN II.

Studying the prevalence of HPV types round the world, meta-analysis was applied (6978 women examined) and it was determined that concerning HSIL, the most frequent type was HPV 16. The prevalence of this type HPV reaches up to 51.8 percent (95% PI: 50.1–53.5) worldwide, while in Europe, it reaches up to 33.3 percent (95% PI: 20.4–48.4) [19]. Variations in the prevalence of other types are not high and usually depend on the geographic area. However, there are some minor variations in HPV prevalence by geographic regions, with variations found mostly in HPV types 16, 18, 33, 45, 31, 58, 52, and 35 [18-20]. Our study revealed that women were mostly infected with HPV type 16 (70.9 percent), 18 (5.1 percent), and 33 (4.3 percent). On the one hand, our estimates of HPV infection and HPV type prevalence confirm already known data.

As far as the HPV function in the carcinogenesis of the cervix is concerned, not only HPV infection and oncogenic HPV types should be mentioned. Virus loads in cells, long-term latent infections, mixed HPV types infections, and the individual's immune system play a significant role. Considering and analyzing the importance of HPV virus as the main cause of cervical cancer, more and more attention is paid to molecular markers, such as virus activity markers (HPV mRNR), gene expressions (i.e., *p16*), and DNA methylation changes [6,7,21]. HPV E2 gene deletion, which means integration of some part of the virus DNA into the host cell genome, is believed to lead to a faster progression of precancerous cervical lesions from minor to major and enhance the probability of developing cervical cancer [22-24].

It is thought that during the integration process (when the HPV DNA integrates into the host cell DNA), virus DNA is disrupted. The viral genome usually breaks in the E1/E2 regions, which usually leads to the loss of the E1 and E2 regions. The deletion of the E2 gene, which encodes proteins, causes loss of transcriptional control

in the E6 and E7 regions. This results in uncontrolled E6 and E7 oncoprotein expression, which causes cell transformation, rapid cell multiplication, and promotes tumor growth. HPV integration into the host genome influences the progression of cervical intraepithelial cells from polyclonal to monoclonal status (CIN). Integrated HPV oncogenes promote malignant tumor cells growth. The opinions and data concerning the relationship between the HPV integration and the level of cervical intraepithelial lesions differ according to various authors. Some researchers suggest that integration of HPV is an early event of carcinogenesis [25,26], while others believe that integration is more frequently detected in already existing significant intraepithelial changes [8,9]. However, there is no doubt about the importance of HPV integration and its influence on cervical changes. Complete virus integration occurs in about 90 percent of cervical cancer cases and is usually associated with oncogenic HPV types [27]. Most of our subjects were infected with HPV 16 and that is the reason why integration (E2 gene deletion) was studied in this type of virus infection.

Eighty-three women were screened for HPV 16 E2 gene deletion. After testing HPV 16 E2 gene separate fragments, mostly a partial (mixed) integration form was detected when one or two out of three gene fragments were identified. Partial (mixed) integration form was detected in 65.0 percent of HPV 16 infected women, complete virus integration in 26.5 percent, and episomal forms in 8.4 percent of cases.

In all confirmed pathological cases (CIN I, CIN II ir CIN III), mostly the partial (mixed) virus integration form was encountered; it was less detected in CIN I cases (60.0 percent), but was more frequent in CIN II and CIN III cases (72.8 percent and 69.3 percent, respectively).

It should be mentioned that virus integration was also detected in cases when cytological changes were not confirmed by histology. Complete integration reached even 40.0 percent, whereas partial (mixed) integration form reached 55.0 percent. Virus integration was found in only 5.0 percent of cases. As was mentioned above, the data in this study corresponds to the research data from other sources which many authors disagree about the stage when the integration of cervical intraepithelial changes occurs. Our study suggests that it might occur at a very early stage, even when histological examination does not show any changes. We failed to answer the question whether viral integration in cervical epithelial cells has implications for the progression of cervical intraepithelial changes because it is known that the development of low grade cervical intraepithelial lesions takes time, while the subjects in our study who had CIN II and higher lesions were treated at that time. However, the progression of minor cervical changes into major changes may develop more quickly in women with virus integration detected early than in those with whom virus integration was not detected. Determination of integration marker (E2 gene deletion) would enable researchers to separate women into a risk group with rapid progression of cervical changes. The results of our study refer to the need of future investigations using a large group of HPV 16 infected women with various cytological changes in the cervix.

5. Conclusions

A percent of 46.3 of women with cervical cytological changes were infected by HPV, with the most frequent type being HPV 16. In all cases of histologically confirmed CIN, mostly the partial (mixed) integration form of HPV16 was identified. It was less frequently detected in CIN I cases (60.0 percent), but more frequently in CIN II and CIN III cases (72.8 and 69.3 percent, respectively).

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