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Frequency of L-SIL and H-SIL Findings in HPV Positive Women

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ABSTRACT

Introduction: Cervical carcinoma is one of the leading health issues for women throughout the world. In 90% of the cases cervical carcinoma develops after development of invasive lesions of the uterine cervix or 10-15 years before they are diagnosed using cytological screening–Papanicolaou test. Infection with human papilloma virus is considered to be the basic etiological cause of development of CIN and cervical carcinoma. **Goal** of this research was to determine frequency and type of abnormal cytological results in HPV positive and HPV negative patients. **Material and Methods:** Research is descriptive analytical, comparative and partly epidemiological, of mostly clinically applicable character. Used data included information on total number of examinations, type of exam, results of cytological and pathohistological diagnostics, HPV findings in cervix smear and HPV type in the period between January 1, 2009 and December 31, 2011. **Results:** During the period of analysis total number of Pap tests was 6376 (43.33%), in comparison to other exams 7828 (56.67%). For this period total number of L-SIL and H-SIL (PAPA III) was 395 (6.20%), in comparison to other results 5894 (92.44%). After analysis largest subset of HPV positive patients had pathohistological diagnosis of CIN I that is 250 (43.10%) of them, then CIN II with 162 (27.93%) patients, CIN III with 149 (25.69) and 3 (0.52%) patients had CIS while 16 (2.76%) had a clear test result. We can conclude there is a statistically significant margin of frequency between positive HPV results in relation to diagnosis. Highest percentage of L-SIL and H-SIL (PAPA III) test results was in 2010 and was 9.5%, and lowest in 2009 with 4.34%. Largest number of patients, 43.0%, with HPV positive results after cervical smear had pathohistological diagnosis of CIN I.

Key words: Cervix, Human papilloma virus.

1. INTRODUCTION

Cervical cancer is one of the biggest health problems of women around the world (1). Cervical cancer is the second most common cancer in women worldwide and is the leading cause of death from malignant tumors in women in developing countries. In the world annually are diagnosed 500,000 new cases of cervical cancer (2). Like many other major diseases, cervical cancer is unequally distributed in the world, over 80% of cervical cancer is detected in developed countries (3).

There is no accurate estimate of the incidence of cervical intraepithelial neoplasia. It is believed that it most often occur in the early thirties, but in recent times cervical intraepithelial neoplasia occur more frequently among young sexually active women (4).

Cervical carcinoma in 90% of cases occurs through the stage, of so-called, preinvasive cervical lesions that occur 10-15 years earlier and are diagnosed by cytological screening method, Papanicolaou test (2). The hypothesis of a viral etiology of cervical cancer, and about its connections with STDs is set almost 100 years ago. In 1976 the theory is restored, and thanks to advances in technology and molecular biology it was possible to isolate the virus, and realize its diversity. Over the past thirty years, scientists around the world have proven that one of the main

causes of cervical cancer is virus called Human Papilloma Virus (HPV) (5). Zur Hausen and colleagues were the first to begin the tests which prove the presence of HPV DNA in cervical cancer cells. Further studies confirmed the association of HPV with malignant changes in other parts of the urinary-sexual and lower intestinal tract: vulva, vagina, anus, penis and prostate (6, 7).

Infection with human papilloma virus is considered a primary etiological cause for the occurrence of CIN and cervical cancer (5, 8, 9). In addition, infection with human papilloma virus represents one of the most common sexually transmitted diseases (10, 11, 12).

2. GOAL

The goal of this research was to determine the frequency and type of abnormal cytological findings in HPV positive and HPV negative patients.

3. MATERIAL AND METHODS

The study was descriptive-analytical, comparative, partly epidemiological, and clinical application mainly character. Used are data on the total number of visits, type of examination, the results of cytological and histological diagnosis, and the finding of HPV cervical smear and HPV typing in the period from Janu-

	2009		2010		2011		Total	
	N	%	N	%	N	%	N	%
Cytological (PAP) smear	2328	44.94	1958	36.96	2090	56.08	6376	43.33
Other examinations	2852	55.06	3339	63.04	1637	43.92	7828	56.67
Total	5180	100	5297	100	3727	100	14204	100

Table 1. The frequency of cytological (Pap) smears in relation to other examinations

	2009		2010		2011		Total	
	N	%	N	%	N	%	N	%
L-SIL; (PAP III)	101	4.34	186	9.50	135	6.46	395	6.20
H-SIL (PAP IV)	25	1.07	20	1.02	15	0.72	27	0.42
Other cytological findings (PAP smear)	2202	94.59	1752	89.48	1940	92.82	5894	92.44
Total	2328	100.00	1958	100.00	2090	100.00	6376	100.00

Table 2. The frequency of the L-SIL and H-SIL (Pap Pap III and IV) findings in relation to other cytological findings (Pap test)

ary 1, 2009 to December 31, 2011. In all subjects had previously done colposcopy examination and Pap test, as well as in patients with abnormal findings underwent HPV cervical smear, and cervical biopsy for histopathological diagnosis.

Diagnostic colposcopy was performed at the Clinic of Obstetrics and Gynecology, Clinical Center of Sarajevo University by Olympus colposcope. In standard colposcopy examination was observed surface of the cervix and vagina vaults after the first flush of mucus and secretions with saline, and then after coating with 3-5% solution of acetic acid under normal lighting conditions, then through a green filter by Kraatz and finally coating with Lugol solution (Schiller's test).

The Pap test is taken from the front and rear lip and cervical canal. From the sample taken was made the cytological smear, which is fixed on a microscopic slide and stained by the method of Pap Nikolai. After staining was performed microscopic analysis of morphological characteristics of the cells in the course of this can detect signs of cervical inflammation, causes infection, benign and malignant cells and other pathological changes.

HPV typing was performed to detect HPV infection, cervical swabs were taken, and then at the Department of Clinical Microbiology, Clinical Center of Sarajevo University was performed HPV DNA PCR test, which is highly reliable, highly specific and sensitive (95-98%). After the punch biopsy of changes in the cervix, the samples were forwarded to the Department of Pathology at the further processing.

The analysis of the results obtained, the following statistical methods were used: for continuous variables in the study the first was used analysis of symmetry (normality) of their distribution by Kolmogorov-Smirnov and Shapiro-Wilk test. For variables with normal distribution and ordinal variables in the study were used χ^2 test, and in the lack of expected frequencies was used Fisher's exact test. The statistical analysis used the Pearson χ^2 test for difference distribution of nominal and ordinal data, and Fisher's exact test with Yates correction in case of lack of expected frequency in comparison variables, the relationship and the direction of the link between changing variables were performed by Spearman's rank correlation test.

4. RESULTS

A detailed analysis of the percentage of performed cytology (Pap) smears in relation to other examinations during the period has been determined that the highest percentage of cytology (Pap) smears in relation to the total number of examinations was in 2011 and amounted to 56.08%, and the lowest in 2010,

36.96%, as shown in Table 1. Non-parametric Mann-Whitney test showed a statistically significant difference in the number of applied cytological (Pap) smears in relation to other examinations annually during the period covered by this study, and in 2011 was made the largest number of cytology (Pap) smears compared to other examinations $Z=-3.678$, $p<0.05$. The frequency of the L-SIL and H-SIL (Pap III) findings in relation to the total number of the performed cytology (Pap) smears was different on an annual basis, as shown in Table 2.

Mann-Whitney test showed a statistically significant difference in the frequency of L-SIL and H-SIL (Pap III) findings in relation to the years covered by this study. In 2010 was recorded the highest percentage compared to the total number of L-SIL and H-SIL (Pap III) findings, $Z=-4.913$, $p=0.004$. The largest number of patients with HPV-positive smear after a biopsy of the cervix had a histopathologic diagnosis of CIN I, 250 (43.10%), and CIN II-162 (27.93%), with CIN III there was 149 (25.69), the CIS had 3 (0.52%), and normal findings had 16 (2.76%) of patients. Pearson chi-square test showed a statistically significant difference in the incidence of HPV-positive smears in relation to the diagnosis, $\chi^2=149.53$, $p=0.000$.

	HPV positive		HPV negative	
	N	%	N	%
CIN I	250	43.10	34	69.4
CIN II	162	27.93	3	6.1
CIN III	149	25.69	3	6.1
CIS	3	0.52	0	0.0
Normal findings	16	2.76	9	18.4
Total	580	100.00	49	100.0

Table 3. Histopathologic findings of cervical biopsy in HPV-positive and negative patients

5. DISCUSSION

Bosnia and Herzegovina does not have a unified register of malignant disease and the number of new cases of cervical cancer per year depending on the size of the tested city or region.

In the study by Ermina Iljazovic (2012) reported that rough incidence of Bosnia and Herzegovina is 26.6. Rough prevalence rate in the region of Sarajevo is more balanced (approximately 30.4), while for the Tuzla Canton this rate varies from 18.5 in the 2005 up to 4.8 in 2000. In the Tuzla canton (the most populated area in Bosnia and Herzegovina), in the period from 1993 to 2006, 27.11% of all women diagnosed with cervical cancer were younger than 30 years, and 1.5% of women even younger than 20 years. Data from Tuzla Canton show a slight increase

in mortality rates over the past five years (4.9) with the peak in 2007 (7.0). In the region of Banja Luka, rough incidence rate of cervical cancer is 24.5, and varies from 15.8 in the 2006 to 28.5 in 2002. Infection with one of the pair of oncogenic types of human papilloma virus (HPV) is a necessary cause of cervical cancer. There is no valid data on the type-specific distribution and the presence of HPV in invasive cervical cancer in most countries, mainly developing countries. The frequency of HPV infection in the general population of women in this region is between 31.37% (2001) and 59.25% (2003). With one stable period during last 6 years (approximately 45%). The incidence of HPV infection in the Sarajevo region is also about 45%, from 47.7% in the 2005 to 45.9% in 2011 (13).

In the course of this research we reached the following results.

By analyzing the frequency of cytologic (Pap) smears in relation to other examinations during the period, led to the data that the highest percentage of cytology (Pap) smears in relation to the total number of visits was during the 2011 year and amounted to 56.08%, and lowest during the 2010 year and amounted to 36.96%. During the covered period the total number of the performed cytological (Pap) smears is 6376 (43.33%), as compared to other examinations 7828 (56.67%). During the observed period (2009-201), we analyzed the frequency of L-SIL and H-SIL (Pap III) relative to the total number of the performed cytology (Pap III) smears. The frequency of the L-SIL and H-SIL varied annually. The highest percentage of L-SIL and H-SIL (Pap III) findings was during 2010 and it was (186) 9.5%, and the lowest in 2009, when it was (101) 4.34%. The frequency of H-SIL (Pap IV) was not statistically different in the observed period. In this period, the total number of L-SIL and H-SIL (Pap III) findings amounted to 395 (6.20%) compared to other cytological findings 5894 (92.44%). Uusküla A. et al (14), in their study, "Incidence of cervical intraepithelial neoplasia in Estonia" stated that the incidence of CIN on 1,000 women is in the range from 0.68 to 2.83 for CIN I, from 0.63 to 1.24 for CIN II and from 0.13 to 0.53 for CIN III.

Rawan A. and Samir A. Saidi (15), in their study "Prevalence of High-Grade Cervical Intraepithelial Neoplasia (CIN) and Cervical Cancer in Women with Post-Coital Bleeding (PCBs) and Negative Smear: A Retrospective Study" noted that in the period from 2005 to 2010, in St. James's University Hospital, Leeds UK, from a total of 1470 patients, 12.1% (179) of them had cervical intraepithelial neoplasia, of which H-SIL 3.8% (56) and 0.4% (6) cervical carcinoma. We conclude that the results for the frequency of L-SIL and H-SIL in the course of our research, are statistically consistent with data from other developed countries.

Analyzing the results of histopathologic analysis in HPV-positive and negative patients has led to the following result.

The largest number of patients with HPV-positive smear after a biopsy of the cervix had a histopathologic diagnosis of CIN-250 (43.10%), followed by CIN II-162 (27.93%), with CIN III there was 149 (25.69), the CIS had 3 (0.52%), and 16 (2.76%) of patients had normal findings. It turned out that there was a statistically significant difference in the incidence of HPV-positive smears in relation to the diagnosis.

In 34 women with HPV-negative findings we found CIN I, CIN II and CIN III in 3 cases retrospectively, while the CIS was not diagnosed. Normal findings were found in nine subjects.

In a study conducted by Kirschner et al. is stated that from

the 290 analyzed cervical biopsy are diagnosed with intraepithelial cervical neoplasia, 276 of them were HPV positive. HPV16 and HPV18 are detected in approximately 75% of cervical intraepithelial lesions of high grade among population in Denmark, and the two genotypes are considered to be the cause of at least 61.9% of cases (16). The study "Correlation of cervical cytology and histology" conducted by Saha et al. (17), 2004-2005, which included 43 patients, 22 of them with benign lesions, 8 LSIL, 9 with HSIL and 1 ASCUS, came to the conclusion that the Pap test is in a statistically significant correlation with biopsy by results.

6. CONCLUSION

The highest percentage of L-SIL and H-SIL (Pap III) findings was during 2010 and it amounted to (186) 9.5% of cases, and the lowest in 2009, where it was (101) 4.34%. The total number of L-SIL and H-SIL (Pap III) findings amounted to 395 (6.20%) compared to other cytological findings-5894 (92.44%). We conclude that the prevalence of H-SIL (Pap IV) was not statistically different in the observed period. The largest number of patients with HPV-positive smear after a biopsy of the cervix had a histopathologic diagnosis of CIN-250 (43.10%), and CIN II-162 (27.93%), with CIN III there was 149 (25.69%) cases, CIS had 3 (0.52%), and normal findings had 16 (2.76%) of patients. In 34 women with HPV-negative we found CIN I, CIN II and CIN III in 3 cases each, while the CIS was not diagnosed. Normal findings were present in nine cases.

Conflict of interest: NONE DECLARED.

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