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ORIGINAL ARTICLE

The relation between human papilloma virus serotype infection and colposcopic, cytological and histopathological abnormalities among the Egyptian women: retrospective study

Short title: Colposcopy, cytology, and histopathology of Egyptian women with HPV

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ABSTRACT

Objective. Correlating Human Papillomavirus (HPV) serotyping, the findings of PAP-smear, colposcopy with colposcopic directed biopsy. Sharing our epidemiological data guides the local health authorities to formalize national screening protocols.

Patient and Methods. A retrospective study was conducted at the Gynecological clinic of a tertiary University Hospital during the period between 2015 to 2020. A total of 285 women's medical records were reviewed for epidemiological, clinical presentation and Pap smear, HPV serotypes, colposcopy findings, and colposcopic guided biopsy. The results were plotted and correlated based on histopathological results.

Results. HPV substantial risk types were detected in 74 (25.8%), and minimal risk serotypes were detected in 28(9.7%). 16 positivity was evident in 13 (12.03%) patients, followed by HPV 31 serotype in 12(11.1%), HPV-51 detected in 11 (10.8%), HPV 18 positivity in 10 (10.02%), while HPV negative in 128 (44.7%). The sensitivity of colposcopy was higher than Pap smear (93.2% Vs 68.52%); however, its specificity was only 69.1% compared to 96.7% of the Pap smear. Our results demonstrated a high agreement between colposcopy and histology 95.36%. similarly, Pap smear and colposcopy agreement was high up to 98 %, but between Pap smear and histology was 84%.

Conclusions. Pairing the result of HPV serotypes with the grade of abnormal cytology, colposcopic appearance, and histopathological findings could improve the early detection of preinvasive lesions.

Key words

Cervical cancer, Cervical biopsy, Colposcopy, HPV serotypes epidemiology, PAP-smear, Egypt.

Introduction

Cancer cervix remains a major health burden worldwide, notably in developing countries that lack both national screening and vaccination programs. It is the 3rd. common cancer among women with around 569,847 new cases and 311,365 deaths in 2018 (GLOBOCAN) [1]. Egyptian cancer Registry statistics declared that: About 969 new cervical cancer cases are diagnosed per annum in Egypt and 631 deaths a year (estimates for 2018) [2]. Although most sexually active females will get an HPV infection at a point in their life, it is self-limited and mostly cleared by the immune system. However, persistent high-risk HPV serotypes infection is the triggering point for the neoplastic intraepithelial changes [3]. These recognizable precancerous cytological abnormalities could help in the early detection of cancer cervix. Nevertheless, most of the national secerning programs combine HPV-based tests and liquid-based cytology tests for patients' triage. HR-HPV 16 and 18 serotypes are the most oncogenic viruses and are detected in 70 % of cervical cancer cases universally [4]. The US Preventive Services Task Force (USPSTF) in 2012 recommended cervical cytology screening every 3 years for women aged 21 to 65 years, with an alternative option for women >30 years for HR-HPV co-testing (cytology and cervical swab for HR-HPV) every 5 years. There is growing evidence that the adoption of primary HR-HPV testing improved the early detection of cervical dysplasia compared to cytology alone [5]. However, studies reported that 10-15 % of women with high-grade cervical cytology are HR HPV negative. Thus, the cervical biomarker role in diagnosing pre-invasive and malignant lesions has

recently increased. These biomarkers such as CEA, SCC-Ag, and CD44, have been used recently not only in the diagnosis of cervical cancer but also in the screening programs and enhance the multidisciplinary approach [6]. Furthermore, Colposcopy is a complementary test with high sensitivity and can guide the gynecologist to target the biopsy of unhealthy cervical epithelium and treat the pre-invasive intraepithelial lesions [7]. The conventional surgical treatment for an early staged cervical cancer is type III open radical hysterectomy with bilateral pelvic lymph node dissection. Consequently, the pre-operative frailty assessment of all patients would personalize the treatment plan and improve the prognosis [8]. Cervical cancer incidence has dropped markedly after the enormous use of vaccination programs worldwide. They protect against HR HPV types 16 and 18: Cervarix (bivalent; GlaxoSmithKline, Belgium) and Gardasil (quadrivalent; Merck and Co., Inc., Whitehouse Station, NJ, USA), both have high safety profiles and efficacy along with cross-protection against another serotype [9]. Yet, HPV being a sexually transmitted virus stigmatize the awareness of vaccination program among adolescent in the middle east, especially in Egypt.

Hence, we plotted the correlation between cytological abnormality, HPV serology, colposcopy, and biopsy findings. Further, we evaluated the prevalence of different HPV serotypes among Egyptian women.

Material and methods

Design: A retrospective observational study following the guideline highlighted in the Observational studies: STROBE (https://www.equator-network.org/reporting-guidelines/strobe/) was conducted at the Gynecological Endoscopy and Cytogenetic unit of Zagazig University Hospital, Al Sharika government, Egypt, during the period between 2015 -2020. The ethical committee of Zagazig university institution has approved this study ZU_IRB ♯65-29-23-11-2020

Sample size: All patients during this period were included.

Methodology

All patients' medical records were reviewed over a five-year duration for all women who attended our gynecology clinic. Inclusion criteria were as follows: women age above 18 years old and presented with contact bleeding or persistent vaginal discharge, or follow-up of previous abnormal cytology. Exclusion criteria: patients with previous cervical surgery and Patients with TZ type 3 because colposcopy will not be satisfactory. Patients with previous hysterectomy or radiation and cervical stenosis.

We recorded the sociodemographic data including residency, age, parity, contraceptive history, menopause status, and smoking. Clinical details were obtained according to the gynecological assessment forum. We reviewed the results of thin preparation cytology taken from the squamocolumnar junction, HPV serotyping, colposcopy findings, and histopathology report.

1. **Hologic Thin Prep smear test (Cytyc).**

Cervical samples were collected by resident gynecology using a cytobrush from the transformation zone (Hologic BVBA, Da Vincilaan 5,1930 Zaventem, Belgium), immersed, and rinsed in a PreservCyt Solution vial. The Thin Prep sample vial was capped, labeled, and sent to a laboratory equipped with a Thin Prep Processor, at Zagazig university, Egypt. The cytological interpretation of the smears was done according to the Bethesda system 2014: (ASCUS) atypical squamous cells of undetermined significance. (LGSIL) low-grade squamous intraepithelial lesions. (HGSIL) high-grade squamous intraepithelial lesions and

(ASC-H) atypical squamous cells cannot exclude high-grade squamous intraepithelial lesions.

2. **Detection of Human Papillomavirus serotype:**

The widely used HVP tests are HC2 (Digene Corporation, Gaithersburg, MD), and AMPLICOR HPV test (Roche Diagnostics, Mannheim, Germany). The HPV serotypes were tested through specimens obtained at the initial screening examination, as mentioned before

LA HPV genotyping test

Initially, PCR was performed in a reaction volume of 100 µl, using 50 µl of LA HPV master mix (Roche Molecular Systems) and 50 µl of DNA. The cycle parameters used were as follows: 2 min at 50°C and 9 min at 95°C; followed by 40 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min; followed by a final extension at 72°C for 5 min and holding at 72°C for up to 4 h before denaturation. The PCR amplicons were denatured by the addition of 100 µl of LA denaturation reagent (Roche Molecular Systems), followed by incubation at room temperature for 10 min. The denatured amplicons (75 µl) were then hybridized and detected using the manufacturer LA protocol. The LA HPV genotyping strips were manually interpreted using the HPV reference guide provided. DNA Pap HC Cervi- Automation of Linear Array sampler (Digene), suspended in 1ml of ViraPap/Viratype transport medium (Digene) and submitted to the laboratory for HPV serotyping by hr-HC2 which included a mixture of probes for both high-risk (HR) cancer-associated HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and the low-risk (LR) probe set for HPV genotypes (6, 11, 42, 43, 44), the test was conducted according to the manufacturer's instructions. In case the result light units/cutoff (RLU/CO) ratio was >1.00, it was considered positive. Regarding, Roche HPV LINEAR ARRAY (Roche Diagnostics, Mannheim, Germany), the LA assay depends on the amplification of a fragment from the L1 region of HPV through using a broad-spectrum PCR primer set, the report of HPV-DNA serotyping was classified as high-risk HPV types (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 82), and low-risk HPV types (6,11, 12, 44,42,61) [10-12]

3. **Colposcopy.**

Women with abnormal cytology results or high-risk HPV serotypes underwent a Colposcopy examination, using a binocular colposcope (Olympus OCSS-BA) with a 40-fold magnification capacity and a green filter. Following visualization of the cervix, a 3–5% acetic acid solution was applied to the cervix using cotton swabs soaked in acetic acid which was prepared by adding 5 ml of glacial acetic acid into 95 ml of distilled water. Cervical epithelial abnormalities could be cellular and or vascular abnormalities. Colposcopic signs of the abnormal cervix; the Inner border sign is a dull, oyster-white area, of the atypical TZ, inside a less opaque acetowhite area, The ridge sign is an opaque mountain-like lesion at the level of TZ, the Rag sign is an abrasion of opaque acetowhite area of the atypical TZ and Cuffed crypt openings are opaque collar-like lesions of acetowhite area at TZ [13].

Biopsy

Different types of colposcopic guided biopsies were applied; a 1-Punch Biopsy was taken from abnormal acetowhite lesions via Townsend Cervical Biopsy Punch Forceps. 2-Multiple biopsies were taken if there were multiple acetowhite lesions. 2- excision biopsy through loop excision of transformation zone electrical wire if big lesions or the whole cervical lip was affected. 3- cone biopsy through cold knife we removed a cone like a biopsy if the lesion was extended to the endocervical canal. Endocervical curettage was done using a Kevorkian curette with a basket rounded tip to collect the product of curettage. Tissue specimens were

evaluated in the Department of Pathology at the University of Zagazig. The report categorized the abnormalities according to WHO classification as chronic cervicitis, cervical intraepithelial neoplasia I (CIN I), CIN II, CIN III, carcinoma in situ, squamous cell carcinoma (SCC), and adenocarcinoma. The slides pathologists were blinded to each patient's cytology, colposcopy findings, and HPV DNA test results [14].

Statistical Analysis & Outcomes:

Data were analyzed using the 25.0 SPSS version (SPSS Inc., Chicago, IL, USA). Descriptive statistics were recorded as numbers and percentages for categorical variables and as mean standard deviation, median, minimum, and maximum values for quantitative variables. In the comparisons of the differences between categorical variables, the Pearson Chi-square test was used.

Results

It showed that the demographic appearance of the population most of them were from urban 88.11 % which reflect better access to health service. Almost half of them were multiparas 54.19 % who encountered symptoms like postcoital bleeding 19.64 % and sought medical advice (**Table 1)**.

HPV DNA was evident in 35.7% (102/285) of women, of whom 34.4% were infected with a single HPV serotype. The prevalence of HR HPV serotypes was 25.9% (predominantly HPV-16 (12.03%), HPV-31 (11.1%), and HPV-51. (10.8%) and HPV-18 (10.02%) compared with 9.08% for any LR HPV type (predominantly HPV-6 (42.5%), HPV-11 (37.5%). Overall, 65.6% (65/102) of HPV-positive women were co-infected with more than one HPV serotype. The most frequent co-infection was associated with HPV-16, 18 (**Table 3).** The HR HPV infection was detected notably among smokers (46.6 %) (**Table 5).**

Discussion

Although cervical cancer is a fully preventable disease, it remains an intrinsic health burden in Egypt. Where early detection of cervical cancer (stage I and/or II) is still exceptionally low (36%) as compared with that in the USA (60%). Unfortunately, various deaths of cervical cancer are not being reported or even diagnosed because of lacking an accurate electronic recording system of the cancer registry [16]. Thus, early detection through screening programs and preventive strategies via vaccination programs are essential to reduce the encumbrance of cervical cancer in Egypt [3]. Eventually, the prevalence of different serotypes gives good insight into the sociodemographic etiopathology of cancer cervix. Consequently, this will improve the management of such cases in line with international guidelines.

The main findings, of our study, explore HPV prevalence and its various type distributions and correlate the diagnostic accuracy of the different diagnostics tools.

According to our study, the prevalence of HPV is estimated to be 35.7% (102/285) of cases, of them, 34.4% had single HPV type serotype infection while 65.6% had multiple HPV serotype infection. **Table 3**

HR HPV type detected in 34.5% (HPV-16 (12.03%), HPV-31 (11.1%), HPV-51. (10.8%) and HPV-18 (10.02%). Meanwhile, LR HPV type was detected in 20.3% (HPV-6 (42.5%), HPV-11 (37.5%). The high-risk HPV was noted more in a younger age than in the low-risk HPV-infected group and was more detected among smoker women too. Interestingly, the overall co-infection rate was around 65.6% (65/102) of HPV-positive women particularly, HPV-16, 18 serotypes. Our results showed an obvious increase in HPV prevalence in Egypt

exceeding the previous study's results (10.3% to 15%) [15]. Concordantly, HPV-16 and HPV-18 serotypes are the most predominant HR HPV types in this study. They are accountable for 61% and 10% of cancer cervix worldwide, and 48% and 23% of cancer cervix in Africa, respectively [11]. This study goes in line with the study of Abdel Aziz MT et al.[15] who reported that the evidence of intraepithelial neoplastic changes on cytological examination was usually associated with a positive HPV DNA status using the HC II assay. Similarly, Ozturk and coworkers [17] concluded that the combination of high-grade cytology changes and positive HR HPV is strongly associated with cervical cancer. This agrees with the study of Sellors and colleagues [18], Carvahalo, and co-workers [19] both reported the presence of high-risk HPV in 100 % of cases of SIL. This supports our assumption of the strong correlation between abnormal cytology and serotyping. It is noteworthy, that CIN 2 & 3 cases with negative high-risk HPV have a good prognosis and less recurrence rate than the corresponding grade with positive high-risk HPV [20].

In terms of the colposcopic findings, we observe that acetowhite lesions were a constant finding among HPV-positive cases ranging from 10% to 42.5 %. while mosaicism and a typical vasculature were more frequently seen with higher-grade cytology in up to 28 % of cases. Our results were concordant with Tidy et al, who reported that acetowhite lesions were frequently detected in colposcopic abnormalities. Cervical cytology is the universal method of screening and early detection of precancerous lesions worldwide, but it has a low sensitivity (66%) and PPV (81%). Colposcopy should be considered as an adjuvant tool that helps to localize the sites of abnormality and target the biopsy sites. The reported Sensitivity is (94.1%) and the specificity was (87.8%) [21]. Our study showed that the sensitivity of Cervical colposcopy was higher than Pap smear (93.2% Vs 68.52%) but its specificity was (69.1% Vs 96.7%). Additionally, it demonstrated a high agreement between colposcopy and histology of 95.36%. while Pap smear and colposcopy agreement was high up to 98 %. But between Pap smear and histology was 84%.

HR-HPV prevalence was found to be higher in the HSIL group up to 71% compared with 34 % in the ASCUS group and only 6 % in the normal cytology group (**Table 4)**. Also, the biopsy confirmed that CIN 3 is more common with a high-grade cytology HSIL compared with low-grade cytology (**Table 2**). This agreement emphasizes the importance of treatment and follow-up cases with HR-HPV to stop the disease progression. also agreed with Liao et al. [22].

Moreover, we reported a high prevalence of some sexually transmitted infections e.g., mycoplasma among HPV-positive women. Thus, we could assume that the vaginal microenvironment might play a role in the persistence of high-risk human papillomavirus (HR-HPV), **Table 4.** This theory has been mentioned in various studies that some mycoplasmas are efficient methylators and may activate carcinogenesis through methylation of (HR-HPV) [23]

Furthered, the prevalence of HPV infection curve was the least in 2014 concomitant with the begging of a national screening campaign and the detection rate of HPV increased gradually with a slight drop after induction of bivalent and quadrivalent vaccines against HR HPV-16, 18, 6, 11 serotypes along with cross-protection against non-vaccine HR HPV-31, 33, 45, 52, or 58. Nevertheless, the increased prevalence of HPV was noted in 2018 due to overpopulation and changes in the sociodemographic parameters of the local community; the increased rate of immigration, multiple marriages, sexual partners, and decreased income affected the health system resources, **Figure 1**.

Area of future development: We could recommend that HPV DNA tests should be offered first for women older than 30 years to overcome the transient nature of HPV infection.

Despite, the high sensitivity of combined screening tools, to assess the real risk of progressive disease, this will add a financial burden on local health authorities. Thus, the solution is to expand a national vaccination program to start with teenagers and offer pap smear screening along with contraception clinics.

The strengths of this study spout from the integrated holistic patient evaluation combing the clinical, cytological, and histopathological diagnosis would be of great value and will add to our regional data registry. Additionally, the pathologists were blinded to PAP test results, indication for colposcopy, smoking habits, and cytological index, so the results were reliable and reproducible. Finally, the .plotted epidemiology of different HPV serotypes could guide the Egyptian ministry of health to design preventive strategies based on sociodemographic distribution. However, the limitations are: Firstly, the inherent bias of the retrospective study, HPV prevalence might change over time due to transient infection. Secondly, we omitted to analyze the lesion's location and size, which can increase the sensitivity to diagnosis. Thirdly, the time between HPV testing and the last sexual intercourse is lacking. Finally, the study was conducted in a single tertiary university hospital in Egypt (Sharkia), not a multicenter study.

Conclusion

There is an existing strong correlation between HPV virus serotype infection, abnormalities of cytology, colposcopy findings, and the grade of the precancerous changes in histopathology. Therefore, the adjuvant use of all these diagnostic tools would improve the early detection rate of cancer cervix. The adoption of customized national screening and vaccination programs ultimately reduces both the morbidity and mortality of cervical cancer. Finally, further research is required to improve information regarding the geographical distribution of HPV types in Egypt.

Compliance with ethical standards

Acknowledgment

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Authors contributions

All authors: Contributed to conceptualization, data curation, investigation, methodology, validation, supervision, visualization, writing— review, and editing.

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Ethical approval

This study had been approved by the ethical committee of Zagazig university: ZU_IRB ♯65-29-23-11-2020

Informed consent

Informed consent was obtained.

Data Sharing

Data are available under reasonable request to the corresponding author.

Disclosure of interests

None of the authors has an actual or potential conflict of interest, including financial and personal relationships with people or organizations within three years of beginning the submitted work that could inappropriately influence (bias) their work.

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Table 1. Demographic association of the studied population.

	F= 285	%Percentage
Age (y)		
(Mean ± SD)	38.1± 8.8	18 -63y
Smoking		
Non-smoker	187	65.7
smoker	98	34.3
Parity		
Nulliparous	131	45.80
Multi para	154	54.19
Residency		
urban	251	88.11
rural	34	11.88
Contraception methods		
➢ **None**	115	40.3
➢ **IUCD**	35	12.2
➢ **COCP**	43	15.8
➢ **POP**	54	18.9
➢ **Injectable**	26	9.12
➢ **condoms**	12	4.21
Gynecological findings		
➢ **normal**	86	30.1
➢ **genital warts**	41	14.3
➢ **vulvovaginitis**	53	18.5
➢ **contact bleeding**	49	17.19
➢ **intermenstrual bleeding**	56	19.64

Table 2. The frequency distribution of the thin prep findings.

Thin repair	F No=285*	Percentage %
➢ **ASUCS**	90	31.1
➢ **ASCUS-H**	2	0.7
➢ **HSIL**	7	2.4
➢ **LSIL**	29	9.8
➢ **Inflammatory**	91	29.7
➢ **Normal cytology**	66	22.0

ASUCS: Atypical squamous cell of undetermined significance. **ASCUS-H:** Atypical squamous cell of undetermined significance high grade cannot be excluded. **LSIL**: low grade intraepithelial neoplasia. **HSIL**: High grade intra epithelial neoplasia.

Table 3. The prevalence of high-risk and low-risk HPV.

HPV prevalence	F	Percentage %	Age Y	P
HPV*				0.24
➢ **Negative**	128/285	44.9	15.5(19.7+_13.3)	CI(0.092 – 0.652)
➢ **HR**	99/285	34.7	16.7 (17.6+_8.9)	
➢ **LR**	58/285	20.3	17.5(21.3+_11.8)	
➢ **Single HPV serotype**	35/102			
➢ **Multiple HPV serotype**	67/102	65.6		
HR				
➢ **HR 31**	12	11.1		
➢ **HR51**	11	10.18		
➢ **HR 16**	13	12.03		
➢ **HR58**	8	7.41		
➢ **HR66**	8	7.41		
➢ **HR 15**	9	8.33		
➢ **HR 12**	2	1.85		
➢ **HR 52**	9	8.33		
➢ **HR 18**	10	10.02		
➢ **HR 59**	6	5.56		
➢ **HR 68**	8	7.41		
➢ **HR 56**	5	4.62		
LR				
➢ **LR 6**	17	42.5		
➢ **LR 12**	7	17.5		
➢ **LR 11**	15	37.5		
➢ **LR 44**	1	2.5		

**MULTIPLE ANSWERS ARE ALLOWED* HR: high risk HPV &LR: low risk HPV

Table 4. Correlation between cytology, HPV serotypes, colposcopic findings, histopathology results, and associated infection.

Thin prep cytology n & %	HPV serotype	colposcopy	Biopsy	Associated infection
Inflammatory, reparative 91/285 (31.9%)	N=91 HR.20/91(21.9%) LR 23/91(25.2%) -ve 48/91(52.7%)	N=10 Normal 5/10(50%) Punctuation 2/10(20%) Mosaicism 2/10(20%) Leukoplakia 1/10(10%) Atypicalvascularity0/10	N=5 Normal 2/5 (40%) Chronic cervicitis 2/5 (40%) CIN 1 1/5(20%) CIN 2 0/5 CIN 3 0/5	N=28 Mycoplasma 13/28(46.4%) Gardenlla 15/28(53.5%)
Normal 64/285(22.4%)	N=64 HR.4/66(6.06%) LR 10/66(15.15%) -ve 50/66(75.75%)	N=4 Normal 2/4(50%) Punctuation ¼(25%) Leukoplakia ¼(25%) Mosaicism 0/4 Atypicalvascularity0/4	N=2 Normal ½(50%) Chronic cervicitis1/2(50%) CIN 1 0/2 CIN 2 0/2 CIN 3 0/2	N=10 Mycoplasma 7/66(10.6%) Gardenlla 3/66(4.5%)
ASCUS **90/285(31.57%)**	N=90 HR.19/90(21.1%) LR40/90(44.4%) -ve 31/90(33.3%)	N=25 Normal 7/25(28%) Punctuation 4/25(16%) Leukoplakia 9/25(36%) Mosaicism 2/25(8%) Atypicalvascularity3/25(12%)	N=18 Normal 5/18 (27.7%) Chronic cervicitis 3/18(16.6%) CIN 1 5/18(27.7%) CIN 2 3/18(16.6% CIN 3 2/18(11.1%)	N=34 Mycoplasma 13/90(14.4%) Gardenlla 21/90(23.3%)
Ascus-H **2/285(0.7%)**	N=2 HR.2/2 100% LR 0/2 -ve 0/2	**N=2** Normal 0/2 Punctuation 0/2 Leukoplakia 2/2 100% Mosaicism 0/2 Atypical vascularity 0/2	**N=2** Normal 0/2 Chronic cervicitis 0/2 CIN 1 0/2 CIN 2 ½(50%)	**N=2** Mycoplasma 2/2 100% Gardenlla 0/2

			CIN 3 ½(50%)	
LSIL **29/285(10.17%)**	N=29 HR.10/29 (34.4%) LR 13/29(44.8%) -ve 6/29 (20.6%)	N=15 Normal 3/15(20%) Punctuation 2/15(13.3%) Leukoplakia 5/15(33.3%) Mosaicism 2/15(13.3%) Atypical vascularity 3/15(20%)	N=15 Repartive3/15(20%) Chronic cervicitis 4/15(26.6%) CIN 1 3/15(20%) CIN 4/15 (26.6%) CIN 3 1/15(6.6%)	N=9 Mycoplasma 6/9(66.6%) Gardenlla 3/9(33.3%)
HSIL **7/285(2.4%)**	N=7 HR.5/7(71.4 %) LR 2/7 (28.5%) -ve 0/7	N=7 Normal 0/7 Punctuation 1/7(14.2%) Leukoplakia 3/7(42.8%) Mosaicism 2/7(28.5%) Atypical vascularity 2/7(28.5%)	N=7 Chronic cervicitis 0/7 CIN 1 2/7(28.5%) CIN 2 2/7(28.5%) CIN 3 3/7(42.8%)	N=7 Mycoplasma 6/7(85.7%) Gardenlla 1/7(14.2%)

Table 5. The correlation between HPV and smoking status.

	Smoker n=98	**Nonsmoker N=187**
HPV*		
➢ **Negative**	46(48.4)	82(43.3)
➢ **HR**	23 (24.3)	47(24.9)
➢ **LR**	20(21.1)	49(25.8)
➢ **Mixed**	8(8.4)	10(5.3)
P value	0.81 CI [0.608 – 1.352]	

Figure 1: The frequency rate of HPV in Zagazig University Hospital across the years.