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Association of Cervical Intraepithelial Lesions in Women with Genital Warts; A Cytological and Molecular Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Cervical cancer is a major health concern worldwide, according to the World Health Organization. It is now the fourth most common cancer after breast, colorectal and lung cancers in women worldwide. It is also the world's third most common cause of cancer death in women. objective of the study is to clarify association between genital warts & cervical intraepithelial lesions using cervical smear cytology with real time PCR.
Methods: The study was carried out at The Department of gynecology & dermatology in Tanta University Hospitals. 50 patients were included started from June 2019 to June 2020.
Results: From June 2019 to June 2020, 50 married women with genital warts are include in the study. Ten women out of 50 were married twice. Number of pregnancies ranged from 0 to 4 with a median (IQR) of 2.56. As regard LSIL group; 6 patients were infected by low risk HPV genotypes while only 2 patients had a mixed infection with high risk HPV genotypes. While all HSIL patients were infected by high risk HPV genotypes.
Conclusions: The cytological characteristics of HPV on cervical smear appear to be non-specific. Therefore, PCR for HPV should be made use of as an adjunct to cervical smears in high risk patients to diagnose and follow up early cervical intraepithelial lesions.

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Keywords: Cervical intraepithelial lesions; genital warts; a cytological; molecular.

1. INTRODUCTION

Cervical cancer is a major health concern worldwide, according to the World Health Organization. It is now the fourth most common cancer after breast, colorectal and lung cancers in women worldwide. It is also the world's third most common cause of cancer death in women [1-2] occurring in developing countries around 80 percent of these deaths. Five out of six women with cervical cancer in developing countries and 80% of them are diagnosed at advanced stage [3].

Without urgent intervention, mortality rate due to cervical cancer expected to rise by almost 25 percent over next 10 years. Cervical cancer is ranked 13th most common among women in Egypt and 10th most common among women between 15 and 44 years of age-[4].

Early detection has resulted in lower incidence and lower death rates. Women treated with precancerous lesions have a 5-year survival rate of almost 100 percent [5-6].

The most important risk factor for cervical cancer is the persistence and lack of timely screening of an oncogenic human papillomavirus (HPV) infection [7-9]. In the early 1980s, German virologist Zur Hausen initially proposed the cause and effect relationship between HPV genital infection and cervical intraepithelial lesions [10].

More than 100 types of HPV have been described, with approximately one third of these mainly infecting genital epithelium. Nearly HPV 6 or 11 (low risk) causing 90% of genital warts and HPV 16 or 18 (high-risk) causing up to 70% of cervical cancers. Therefore, only 4 HPV serotypes are responsible for most of the HPV disease burden [11]. It is estimated that approximately 20% to 50% of people with low-risk infections may also have co-infections with what is known as high-risk types [12]. In a study on the prevalence of HPV infection among high risk group of Egyptian women, PCR of cervical biopsies revealed an approximately 70% infection rate [12]. but no data are available on the infection rate on general population [13].

Clinically, HPV infects the basal cells of the epithelium [14] of skin and mucous membranes [15]. The majority of genital warts are described as cauliflower-like condyloma acuminata, or genital papillomas. Genital warts can also be dome shaped, papular, pedunculated or flat & they can occur as single lesions, in clusters or as plaques [16].

Genital warts can cause intense discomfort with associated pruritis, bleeding, and secondary infection caused by superficial injury due to scratching [17]. The latent infection with this virus can cause dysplasia, neoplasia, and cervical cancer. The difference in age of peak HPV infection and peak of cancer incidence is two to four decades, making screening an ample area for innovation and progression toward improved disease management [18-19].

objective of the study is to clarify association between genital warts & cervical intraepithelial lesions using cervical smear cytology with real time PCR.

2. PATIENTS AND METHODS

The study was carried out at The Department of gynecology & dermatology in Tanta University Hospitals. The duration of the study was 12 months, started from June 2019 to June 2020. Fifty patients were included in this study. The sample size was calculated using Epi-Info software statistical package created by World Health organization and center for Disease Control and Prevention, Atlanta, Georgia, USA version 2002. The criteria used for sample size calculation (n>33) were 95% confidence limit, 80% power of the study, expected outcome in in treatment group 90% compared to 60% for control groups.

2.1 The Inclusion Criteria

1. Adult married women ≥18 years of age
2. Presence of genital warts.

2.2 The Exclusion Criteria

1. Virgins.
2. Conditions that may alter specimen taking, e.g., pregnancy.
3. Active vaginal bleeding.

The details of the investigations done, technique and complications were explained to the patients and an informed consent was obtained.

2.3 All Patients Subjected to the Following

1. Personal history (name, age, address, telephone number, marital status, number of pregnancies).
2. High risk practices (smoking either passive or active, unprotected sex).
3. Present history: history of onset, course and duration of the genital wart.
4. Sexual history (number of marriages, age of first sexual contact, usage of condom, multiple sexual partners).
5. Intake of hormonal contraceptives, gynecological history, previous pap smears.
6. Past history: history of medical disorders, drug therapy.
7. Family history: history of any similar conditions.
8. Perineal inspection of genital warts site, number.

2.4 After Selection of the Cases

The woman was prepared in the usual manner of cervical smear by asking her to lie in the lithotomy position. Non lubricated vaginal bivalve speculum was inserted. The cervix, vagina, vaginal wall and fornices were carefully inspected for the presence of any pathological lesion, and then endocervical and ectocervical samples were collected from each woman using an Ayre's spatula or tongue depressor, after cleaning the cervix using sterile cotton swab. Sampled material will smoothly spread over clean, labeled slide and fixed with 95% ethyl alcohol, for cytological examination. Positive for high grade cervical smear cell sample was assigned for molecular study (HPV DNA testing using real time PCR).

Sample preparation for cytological examination: The cervical smear cell samples, preserved in 95% ethyl alcohol were stained by haematoxylin & eosin (H&E) stain for microscopical examination, diagnosis and pathological grading according to Bethesda system 2001.The slides were subsequently reported as NILM, ASCUS, LSIL, HSIL or carcinoma.

Preparation of PCR specimen: Spatula parts, after being cut from the shaft, were immediately put into specimen transport 1.5 ml plastic tube containing 500 µl of sterile 0.9% sodium chloride. All tubes were transported at 4ºC to the PCR Laboratory where they were stored frozen at -20ºC until tested for HPV DNA.

2.5 Statistical Analysis

The criteria used for sample size calculation (n>33) were 95% confidence limit, 80% power of the study, expected outcome in in treatment group 90% compared to 60% for control groups.

Quantitative parametric variables (e.g. age) were presented as mean and standard deviation (SD). Quantitative non-parametric variables (e.g. VAS) were presented as median and range and compared between the two groups by Mann Whitney (U) test and within the same group by Wilcoxon test

3. RESULTS

This study samples were collected randomly from 50 married women with ages between 30 to 45 years visiting Gynecology clinic of Tanta University. Samples were tested microscopically for cytological changes; selected cases were tested by PCR for detecting HPV genotypes. Women living in rural areas are 32 (64%) patients while those living in urban are 18 (36%). Only 3 women were active smokers & 15 were passive smokers. Six women were diabetic (12%). None of the women had a positive family history of genital warts.

As regard mean age of NILM and ASCUS is 35 years old while mean age of LSIL and HSIL is 43 years old. A total of 38 patients were having intercourse without using condom had a NILM or ASCUS smears whereas 8 patients had an abnormal cervical smear in form of LSIL or HSIL. Hormonal contraceptives were used by 25 cases with normal to inflammatory cervical smears in comparison with 5 cases of squamous intraepithelial lesions.

As regard smoking; 3 patient was actively smoking. None of both groups having a positive family history of cervical cancer.

Duration of infection of genital warts as early as 3 months up to 21 months with episodes of cure and relapse. In majority of cases; warts are multiple ranging 1 to 6 warts in single or multiple areas.

Statistical study showed significant correlation ($P<0.05$) between age and cervical lesions; as

NILM and ASCUS were present in younger age group if compared to LSIL and HSIL age group.

3.1 The Cytopathological Examination of These Smears by H&E According to Bethesda System 2014

1. A total of 30 cases (60%) were NILM (inflammatory): predominance of superficial squamous cells admixed with few intermediate and parabasal ones without atypia. A range of mild to heavy inflammatory cells mainly neutrophils.
2. While 9 cases (18%) were ASCUS: sheets of few atypical metaplastic squamous cells showing mild nuclear enlargement and pleomorphism with clear perinuclear halos. Infrequent inflammatory cells.
3. A number of 8 cases (16%) were LSIL: sheets of moderate atypical squamous cells with hyperchromatic enlarged nuclei and irregular nuclear contour
4. And only 3 cases (6%) were HSIL: sheets and groups of highly atypical small squamoid cells, high N/C ratio, nuclear hyperchromatism and irregular nuclear contour.

Table 1. Demographic characteristics, special habits and family history of the studied sample

		All patients (n= 50)
Age (years)		38.00 ± 4.347
Residency	Rural	64.0% (32)
	Urban	36.0% (18)
Smoking	None smoker	64.0% (32)
	Active	6.0% (3)
	Passive	30.0% (15)
History of DM		12.0% (6)
Family history of same condition		0.0% (0)

Data is expressed as mean and standard deviation or as percentage and frequency.

Table 2. Sexual history of the studied patients

	All patients (n= 50)
Number of marriages	1.30 ± 0.463
Age of first sexual contact (years)	22.80 ± 2.556
Intercourse without condom	92.0% (46)
Intercourse with condom	8.0% (4)
Hormonal contraceptives	60.0% (30)
Number of pregnancies	2.56 ± 0.861

Data is expressed as mean and standard deviation or as percentage and frequency.

Table 3. Age, hormonal contraception, smoking and family history distribution in studied cervical lesions in the current study

		NILM, ASCUS	LSIL, HSIL	P
Age		36.44 ± 3.553	43.55 ± 1.128	< 0.001
Intercourse without condom		97.4% (38)	72.7% (8)	0.029
Hormonal contraceptives		64.1% (25)	45.5% (5)	0.265
Smoking	No	71.8% (28)	36.4% (4)	0.067
	Active	5.1% (2)	9.1% (1)	
	Passive	23.1% (9)	54.5% (6)	
Family history		0.0% (0)	0.0% (0)	-

Data is expressed as mean and standard deviation or as percentage and frequency. P is significant when < 0.05.

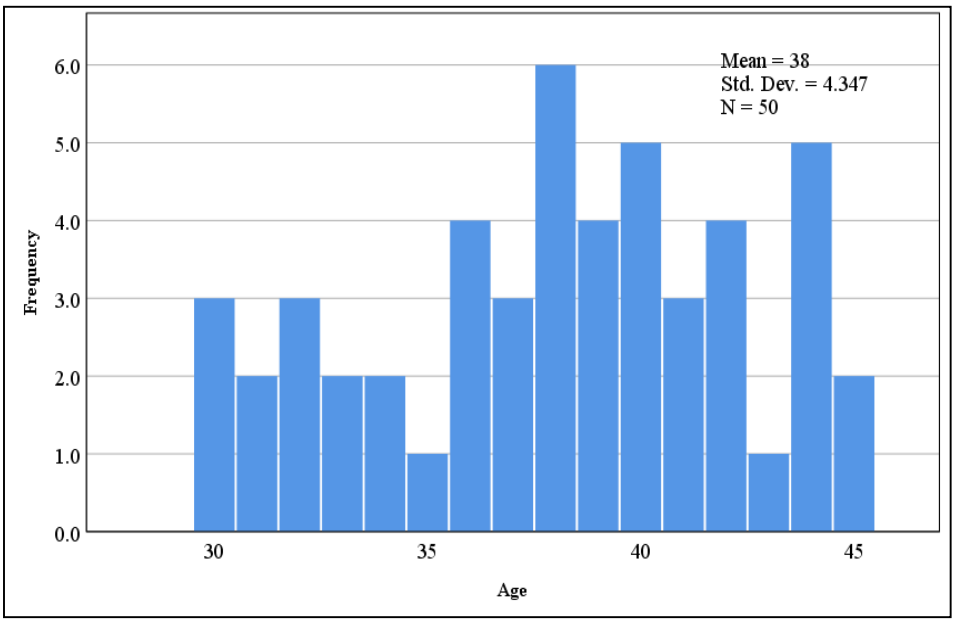

Fig. 1. Age distribution of the studied sample

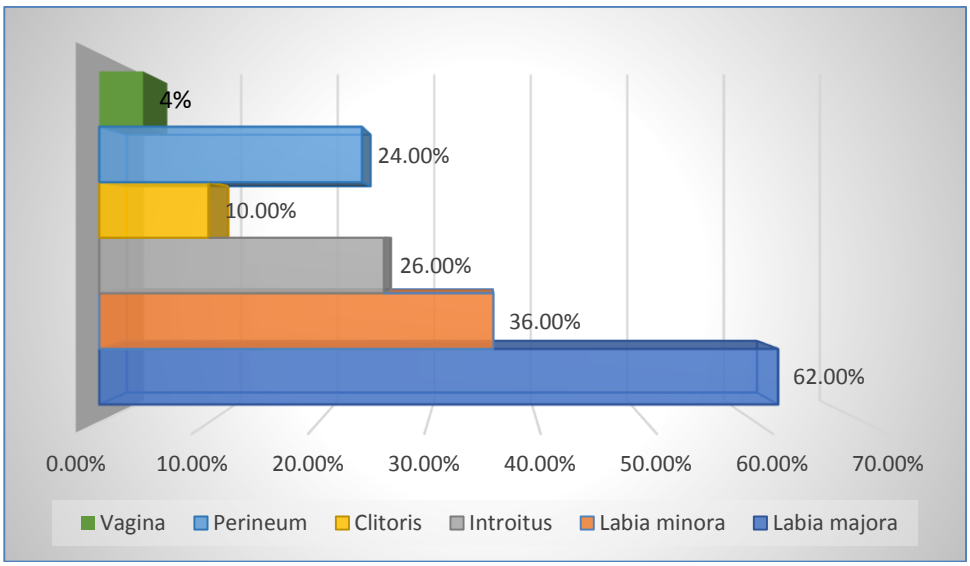

Fig. 2. Location of genital warts in the current study

Table 4. Duration, number and location of genital warts in the current study

		All patients (n= 50)
Duration (months)		11.84 ± 5.358
Number		3.34 ± 1.547
Location	Labia majora	62.0% (31)
	Labia minora	36.0% (18)
	Introitus	26.0% (13)
	Clitoris	10.0% (5)
	Perineum	24.0% (12)
	Vagina	4.0% (2)

Data is expressed as mean and standard deviation or as percentage and frequency.

HPV DNA was extracted from cervical swab taken from 11 patients. Viral DNA was identified; Table 3 shows the distribution of types. Six patients (54%) were infected with single HPV genotype while 5 patients (46%) infected with multiple genotypes. The commonest HPV genotypes associated with intraepithelial lesions were HPV 11; 6/11 (54.5%), HPV 16; 3/11 (27%), HPV 6; 4/11 (36%); LSIL are mostly of single genotype while HSIL are in the form of multiple infections.

As regard LSIL group; 6 patients were infected by low risk HPV genotypes while only 2 patients had a mixed infection with high risk HPV genotypes. While all HSIL patients were infected by high risk HPV genotypes.

Table 5. Cervical intraepithelial lesions in the current study

		All patients (n= 50)
Cervical smear	NILM	60.0% (30)
	ASCUS	18.0% (9)
	LSIL	16.0% (8)
	HSIL	6.0% (3)

Data is expressed as percentage and frequency.

Table 6. Genotypes and result of PCR examination of samples with cervical intraepithelial lesions in the current study

			All patients (n= 11)
Type	Single genotype		54.0% (6)
	Multiple genotypes		46.0% (5)
Genotype	LSIL	11	50% (4)
		6	25% (2)
		6 & 16	12.5% (1)
		11 & 18	12.5% (1)
	HSIL	11 & 16	66.7% (2)
		6 & 16	33.3% (1)

Data is expressed as percentage and frequency.

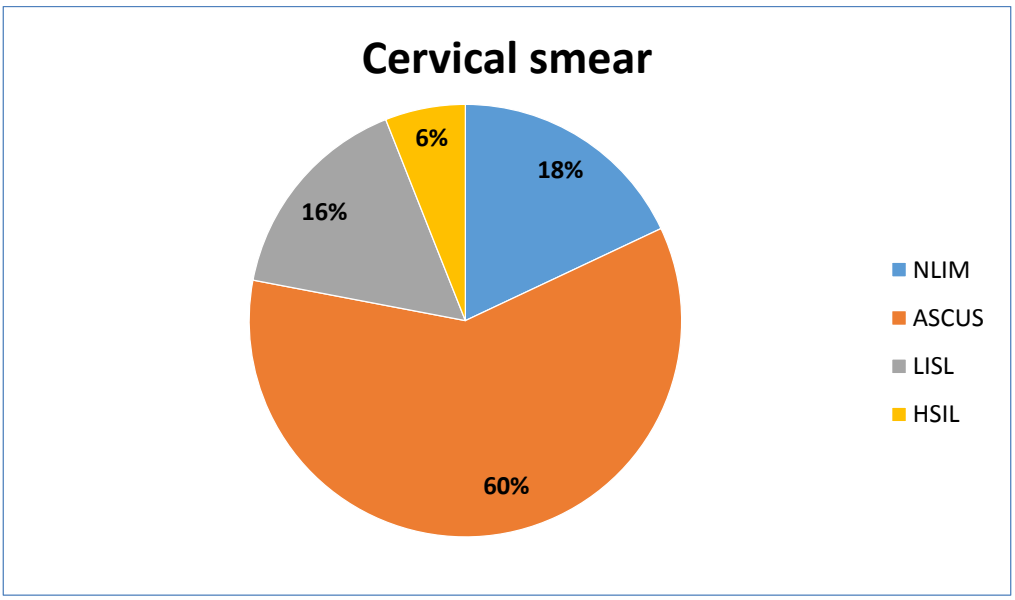

Fig. 3. Cervical smear results in the current study

Table 7. High and low-risk serotypes in LSIL and HSIL in the current study

	Low risk	High risk	P
LSIL	6	2	0.061
HSIL	0	3	

Data is expressed as percentage and frequency. P is significant when < 0.05.

Shape, location & numbers of some of genital warts which were reported in the study:

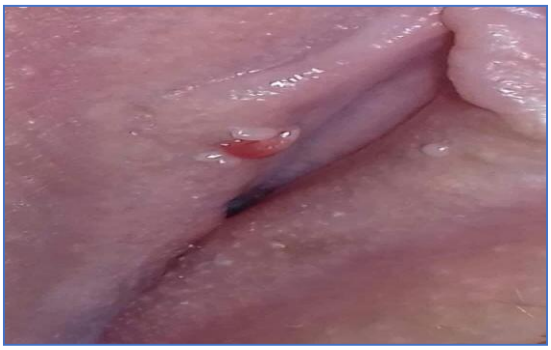

Fig. 4. Multiple genital warts on introitus of vagina

Fig. 5. Groups of genital warts on labia majora

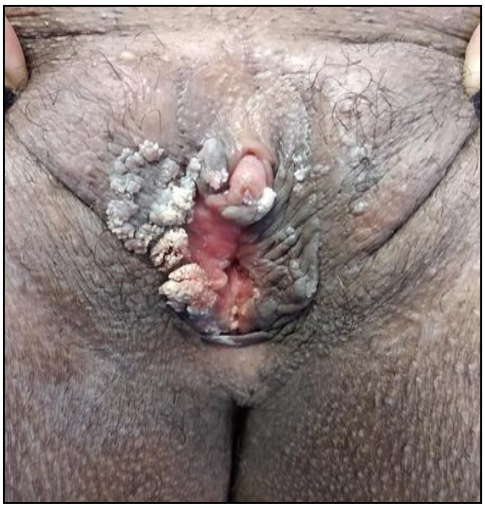

Fig. 6. Groups of genital warts on labia majora, minora and paraurethral area

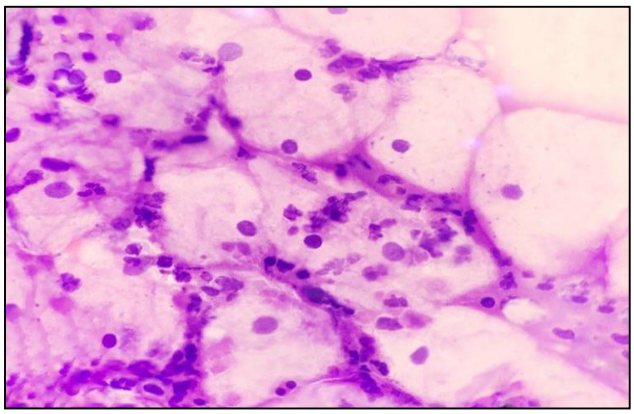

Fig. 7. NILM; smear showing superficial and intermediate squamous epithelial cells with an inflammatory background. (H&Ex400)

4. DISCUSSION

HPV is established as a public health problem for its role as an essential factor in pathogenesis of various cancers. Persistence of oncogenic HPV seems to be essential for the occurrence of cervical neoplasia [20]. The malignant transformation activity of HPV-16/18 is well-established. With the arrival of molecular techniques, especially PCR, it is possible to detect commonly occurring HPV types in cervical smears. The cytological characteristics of HPV on cervical smear seems to be non-specific [21]. Therefore, HPV testing should be done as an adjunct to cervical smears.

Most of cervical cancer cases arise from SIL, but not all reported lesions progress to cancer. Actually, many persist without change or even regress [18]. It is believed that CIN I and II are more likely to regress than to progress; only 10.0%-15.0% of CIN I lesions are progress to CIN II and III/CIS, and 50.0% of CIN II and 30.0% of CIN III regress spontaneously [22].

Egypt has a population of 30.55 million women aged 15 years and older who are at risk of developing cervical carcinoma. Current estimates indicate that every year 866 women are diagnosed with cervical cancer and 373 die from the disease. Cervical cancer in Egypt ranks the 13th most frequent cancer among women between 22 and 44 years of age [23].

The majority of studies done up to now have looked at DNA levels, whereas the study done by Gnanamony *et al.,* 2009, in India, showed that active replication, as seen by an increasing mRNA transcript level and not DNA levels, can be a marker of progressing cervical disease. Gnanamony *et al* 2009, estimated the DNA and mRNA viral loads of the most common high-risk HPV 16 and 18 in cervical biopsy tissue of patients with cervical neoplasia via real-time PCR [24].

In the present study, the age of patients ranged from 30 to 45 years with median (IQR) of 38 (+/-4.347) years of women with genital warts attending Gynecological examination.

Studies from different regions of the world such as Greece have reported an overall HPV prevalence ranging from 22.7 to 49.1 % [25] in women aged 16-45, 16-71 years respectively. Kuhn et al. [26] in the USA, reported that HPV-positive rates of 22% within age distribution 35-65 years; it was lowest in women aged 40-49 years. Sellors et al. in Canada; revealed that prevalence of HPV was 24% in age group of 20-24 years & 3.4% in older age group of 45-49 years [27] Sun et al. [28] in Taiwan reported 21 %, respectively. On the other hand, Varghese et al. [29] reported 6.1 % among Indian women. This augments the findings of Becker et al. [30] that there are regional and ethnic differences in HPV prevalence.

Of the 50 women referred, 11 had histological evidence of intraepithelial lesions (8 had LSIL, and 3 had HSIL), 9 had evidence of ASCUS, and 30 had (NILM). This study showed that mean age of women with intraepithelial lesions is 43 years while NILM and ASCUS is 36 years which showed significant statistical difference ($P<0.05$). This was consistent with what is shown that women with CIS/cervical cancer are older than those with dysplasia [31] so progression of malignant changes in cervical epithelium is a function of age [32]. Data reported that 70.5% of

the CIN II or CIN III (HSIL) lesions are found in women aged 30-40 [33]. The cancer incidence rate increased after age 30 and peaked in 55-65 [34].

This study showed 11 patients out of 50 (22%) with established intraepithelial changes at initial screening confirms the conclusions of Walker et al that women with genital warts are at high risk of having CIN [35]. These findings support the view that women attending for the first time with genital warts required screening for cervical intraepithelial neoplasia.

This study couldn't show any differences between women with intraepithelial lesions and those with normal cervices at initial screening in respect of sexual behavior, smoking habit, oral contraceptive use. These results are coincident with Youssef et al., (2016), Schmeink et al., (2010), and Demir et al., (2012). On the other hand, Abdel Aziz M.T. et al., (2006), Irimie et al., (2011), Thabet et al., (2014) and El-Moselhy et al., (2016) have shown a statistically significant relation between HPV infections and contraception. Walkinshaw et al.,(1988) who studied a larger group of women with CIN and cervical HPV, suggested that women with CIN who smoked were heavier smokers than those with HPV infection only without cervical intraepithelial lesions [36]. Our study did not confirm this finding may be due to cultural traditions and type of population. The larger study did not include data on duration of smoking. Other studies implicating oral contraceptives or smoking in cervical cancer used "normal" women as controls and did not give data on past or current genital warts [37].

Lesions containing high risk genotypes HPV 16 and 18 are more likely to progress to high grade intraepithelial lesions [38].

5. CONCLUSIONS

The cytological characteristics of HPV on cervical smear appear to be non-specific. Therefore, PCR for HPV should be made use of as an adjunct to cervical smears in high risk patients to diagnose and follow up early cervical intraepithelial lesions...

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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