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ORIGINAL ARTICLE

Prevalence of HPV 16 and HPV 18 in cervical cancer in Minia Governorate

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ABSTRACT

Background*: Human Papilloma virus (HPV) have a critical role in cervical cancer especially type 16 and 18 and great efforts were done to determine the role of HPV in cervical cancer.* ***Objective****: The objective of this work is evaluation of the prevalence of cervical cancer and determination of the role of HPV in cervical cancer, in Minia Governorate.* ***Methodology****: Colposcopic-directed biopsy was done for one hundred females by colposcopist. One hundred women were included in this study for detection of HPV infection by histopathology, real time PCR and sequencing. One hundred biopsies were subjected to analysis for detection of HPV types 16 and 18 by histopathology, real time PCR and sequencing.* ***Results and conclusion:*** *Our histopathological results revealed that 15% of samples were positive for HPV and by HPV DNA real time PCR, 14% were positive. Our study reported that the infection was single in 9% and mixed in 5% and HPV type 16 was higher than type 18. The age range across the sample was 18–55 years with a median age of 35 years. The women patients with subtype 18 had the highest median age. Contraceptive usage had a statistically significant difference especially using OC.*

INTRODUCTION

Each Human Papilloma virus *(HPV)* has its own number or type. *HPV* is a family Papovaviridae double-stranded DNA virus. With more than 40 forms colonizing the genital tract, nearly 200 forms of *HPV* have been reported. *HPV* lives in small, flat cells known as epithelial cells which are located on skin surface. They're also present on the vagina, uterus, anus, vulva, cervix and penis head. They're found inside the mouth and throat too [1].

Around 60 % *HPV* cases trigger warts on areas as the hands or feet. The remaining approximately 40 % enter the body during sexual activity. They're drawn to the mucosa membranes of the body, like the moist layers covering the anus and genitals. Not all of the 40 % sexually transmitted HPV viruses cause severe health concerns but are listed as high-Risk and low-Risk groups [2].

High-risk *HPV* strains include *HPV* types 16 and 18, which account for about 70% of cervical cancer. Many *HPV* viruses at high risk like 31, 33, 45, 52, 58. Low-risk *HPV* strains, including *HPV* 6 and 11. Such upheavals can sound like bumps. They are shaped as cauliflower [1].

According to latest estimates by World Health Organization (WHO), in Egypt, 866 women are diagnosed with cervical cancer every year and 373 die from the disease. Cervical cancer is the 13 th most common cancer in women in Egypt and the 10 th most common cancer in women aged between 15 to 44 years[3].

Statistics from Egyptian studies provide pre invasive cervical lesion incidence levels of 0.3 % to 0.5 % [4].

Cervical cancer (CC) is caused by forms of *HPV* strains belonging to a few HR species (5, 6, 7, 9,11).The forms most commonly in CC (-16, -18, -31, -33, -35, -45, -52, -58) and four less-common types (-39, -51, -56, -59). The remaining forms of *HPV* have been listed as potentially carcinogenic [5].

There are currently two vaccines available in several countries around the world for protection against HR *HPV* types 16 and 18, as Egypt,: Cervarix (bivalent; GlaxoSmithKline, Belgium) and Gardasil (quadrivalent; Merck and Co., Inc., Whitehouse Station, NJ, USA). Both vaccines have good profile of safety and efficacy and are confirmated to provide cross-protection against non-vaccine HPV forms. Although both of these vaccines are already approved in Egypt, they are currently part of the national immunization program [6]. The objective of this study was evaluation of the prevalence of cervical cancer and determination the role of *HPV* in cervical cancer, in Minia Governorate, Egypt.

METHODOLOGY

Patients:

The present research was performed on 200 patients presenting at the female Outpatient Department at Gynecology and Obstetrics Minia University hospital

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from July 2018 to December 2019. It performed in collaboration with Departments of Pathology and Microbiology. Two hundred females in the reproductive age group showing unhealthy cervices by per speculum test were subjected to a comprehensive history, clinical review and only one hundred cases showed abnormal cervix.

Specimens:

Colposcopic-directed biopsy was done for one hundred females by colposcopist. After detailed examination of the transformation zone, punch biopsies were taken from any suspicious region. For histopathological examination, the specimens were fixed in 10% buffered formalin for evaluation by the Pathology Department at Minia University. Biopsies were performed with punch biopsy forceps. If the cervix had more than one suspicious area, biopsies were picked up separately, and the diagnosis of each case was graded according to the highest histopathological grading of any area.

Mapping of positive results on special graph were done. All cervical biopsies were interpreted using standard histological descriptions into negative (normal or inflammation), CINI, and CIN II+ (including CIN II, CIN III, and early-stage squamous cervical carcinoma).

Questionnaire

At recruitment, all women completed the ethnic group, marital status and contraceptive status questionnaire.

Ethical considerations

The study was approved by the Ethics Committee of the faculty of medicine, Minia University. Informed consent was obtained from all eligible women before starting the study.

Detection of *HPV*:

Histopathological Examination: diagnosis of *HPV* was based mainly on the presence of dysplastic cells with the HPV characteristic appearance. The characteristic appearances of HPV infected cells were koilocytosis, binucleation, dyskeratosis, basal cell hyperplasia, papillomatosis and Acanthosis. The koilocytic cells nuclei were surrounded by extensive clear halo (koilos), giving the cells their name. The cytoplasm was condensed at the cell periphery around these clear zones. The superficial dyskeratotic cells had dark, hyperchromatic nuclei and orangeophilic cytoplasm, signifying a disturbance in their terminal differentiation (dyskeratosis). Even in the absence of koilocytes, such cells in young women are highly suggestive for *HPV*, albeit not entirely conclusive.

HPV DNA analysis by Polymerase Chain Reaction: (Reliance Life Sciences), DNA was extracted from cells using a Qiagen DNeasy Kit and used as positive controls in the real time PCR assay. Paraffin-embedded patient tissue was deparaffinized with xylene. Tissue was washed twice by using 100 percent ethanol and dried. DNA extraction using a Qiagen DNeasy kit as instructed by the manufacturer's (Qiagen, 2006, Germany).

PCR was carried out in 20 µL reactions using 12.5 µL of PCR master mix (Nippon genetics, Europe), 1 µL (12.5 pmol) of each primer, 1 µL (12.5 pmol) of each Probe, 2µL (20 ng) of template DNA and 3.5 µL of sterile water. The thermal cycle conditions were an initial denaturation incubation at 95°C for 10 minutes followed by 40 cycles of alternating 95°C incubations for 15 seconds and 60°C incubations for 30 seconds. Quantitation of PCR products was performed using applied biosystem software 7.

Table 1: Primer and probe [8]

1	HPV16L1 (F)	TTGTTGGGGTAACCAACTATTTGTTACTGTT	400nM
2	HPV16L1 (R)	CCTCCCCATGTGAGGTACTCCTTAAAG	400 nM
3	HPV 16L1 probe	6FAM-GTCATTATGTGGTGCCATATCTTCACT- TAMRA	400 nM
4	HPV 18L1 F	GCATAATCAATTATTTGTTACTGTGGTAGATACCACT	400Nm
5	HPV 18L1 R	GCTATACTGCTTAAATTTGGTAGCATCATATTGC	400Nm
6	HPV 16L1 probe	AACAATATGTGCTTCTACACAGTCTCCTGT	

Sequencing: 3 microliters of ExoSAP-IT (USB Corporation) were added to 7 microliters of positive reactions generated by PCR assay. Reactions were incubated at 37°C for 15 minutes followed by an incubation at 80° for 15 minutes. For DNA sequencing, the entire volume was used using HPVE1F as a sequencing primer (Eton Bioscience Inc.). Blast sequence analysis was performed on generated sequences to identify homologies with other known *HPV* DNA.

Statistical analysis:

The statistical analyses were undertaken using SPSS version 9.2

RESULTS

In our study, 200 female were tested for any abnormal cervix and after evaluation, only 100 cases were included in the study for HPV infection by

histopathology, real time PCR and sequencing, The Mean± SD of the age of our patients was 32.8±8.5.
The baseline characteristics of women enrolled in the study are shown in Table 2.

Table 2: The baseline characteristics of the cases

N=100		N	%
Contraception use	NO contraception use	25	25.0
	Oral contraception (OC)	31	31.0
	Intrautrine Device(IUD)	44	44.0
Marital status	Married	92	92.0
	divorced	8	8.0
Residence	Urban	64	64.0
	rural	36	36.0

By colposcopic-directed biopsy, the histopathological results revealed that 85 % patients were negative for *HPV* infection while 15% were positive for HPV and by *HPV* DNA real time PCR , 14% of the cases were positive for *HPV*. The results were showed mixed infection with the two types HPV 16 and 18 in 5% of cases while 9% of cases were infected with a single type (5 % and 4 % for HPV 16 and 18 respectively) as shown in table 3. sequencing were done for only 5 cases positive *HPV* DNA PCR and the results revaled the same result as PCR

Table 3: Pathology and PCR positive patients

N=100		N	%
Pathology	positive	15	15.0
	Negative	85	85.0
PCR	Negative	86	86.0
	16	5	5.0
	16/18	5	5.0
	18	4	4.0

Comparison between pathology results regarding age of patient showed the mean was 72±53 as shown in table 4.

Table 4: Pathological changes to the age group

Pathology	N	Mean Rank	Sum of Ranks	Median	U	P
Negative	85	46.61	3962	29	307.0	0.00*
Positive	15	72.53	1088	43		
Total	100			31		

N.B:A Mann-Whitney U revealed significant difference of median age of positive group (Md=43) and negative group (Md= 29) (U= 307.0 , P= 0.001)

Comparing the pathology result with other baseline characteristics showed that the contraception usage had a significant statistical difference. Usage of oral contraceptive were 40%, 26.6% for IUD and 33,3% for non contraception users. Regarding Marital status and residance there were no significant statistical difference as shown in table 5.

Table 5: Relation of pathological changes to baseline characteristics

		Pathology		Significance
		Negative n(%)	Positive n(%)	
Contraception	No	20 (23.5)	5(33,3)	χ^2= 6.9 P= 0.031*
	OC	25(29.4)	6(40.0)	
	IUD	40 (47.0)	4 (26.6)	
Marital status	Married	79 (92.9)	13(86.7)	χ^2= 0.7 P=0.41
	divorced	6(7.1)	2 (13.3)	
Residence	Urban	53 (62.4)	11(73.3)	χ^2= 0.7 P=0.41
	Rural	32(37.6)	4 (26.7)	

Comparison between PCR results and the age of patients among the study participants, revealed a statistically significant difference in the age group across different PCR results (P=0.001). Patient with subtype 18 had the highest median age (Md=48.5) across all subtypes and negative cases as shown in table 6 and figure 1. And the distribution of age group and HPV infection reported the highest rate lies in age above 50 years as shown in table 7.

Table 6: Relation of positive cases by PCR and age group

	PCR	N	Median age	KW	P
Age	Negative	86	29.5	17.098	0.001*
	16	5	45		
	16/18	5	40		
	18	4	48.5		
	Total	100	35		

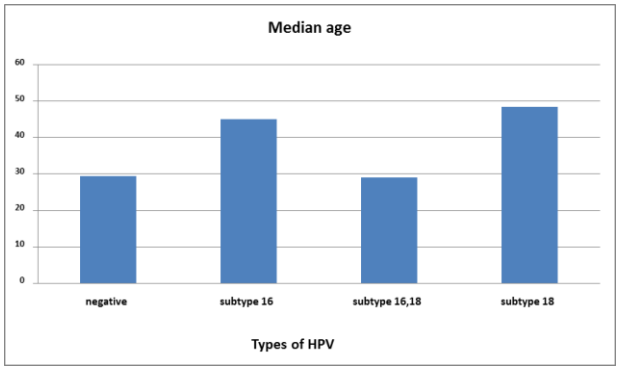

Fig. 1: Relation of age groups and PCR positive cases

Table 7: Distrubation of age group among HPV positive cases

Age groups	*HPV*	
	No	%
18-28 years	0	
29-39 years	2	14.2
40-50 years	5	35.8
> 50 years	7	50

Association of PCR results and contraception usage reveled a statistically significant difference as HPV type 16 was detected in 2(50%) of cases using OC, 1(25%) using IUD and 2(50 %) that did not used contraception methods. Mixed infections with type 16 and 18 were reported in 2(50%) using OC and 1(25%) using IUD and 1(25%) that did not used contraception methods while infection with type 18 were reported in 1(25%) using OC and 2(50%) using IUD and 1(25%) that did not used contraception methods as shown in table 8.

Table 8: Relation of positive cases by PCR and contraception methods

Contraception	PCR								Total		Significance
	Negative		16		16/18		18				
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	
NO	0	(0.0)	1	(25.0)	1	(25.0)	1	(25.0)	3	(21.4)	Fisher exact = 3.86 P=0.01*
OC	1	(50.0)	2	(50.0)	2	(50.0)	1	(25.0)	6	(42.9)	
IUD	1	(50.0)	1	(25.0)	1	(25.5)	2	(50.0)	5	(35.7)	
	2	(100.0)	4	(100.0)	4	(100.0)	4	(100.0)	14	(100.0)	

Our study showed that there were 2 negative cases by real time PCR method and positive by pathology and one case was positive by real time PCR and negative by pathology as shown in table 9.

Table 9: Relation by PCR and pathology

		Pathology				Significance
		Negative		Positive		
		N	(%)	N	(%)	
PCR	Negative	84	(98.8)	2	(13.3)	Fisher exact =56.7 P= 0.0001*
	16	0	(0.0)	5	(33.3)	
	16, 18	1	(1.2)	4	(26.7)	
	18	0	(0.0)	4	(26.7)	

DISCUSSION

Nevertheless, *HPV* infection rates continue to occur, particularly in developing countries, which still have a high rate of incidence and prevalence of cervical cancer. It is attributed to various factors including low socioeconomic status, lack of public awareness, and insufficient screening and vaccination system [9].

HPV cannot propagate in tissue culture and therefore, molecular biology is used to correctly identify it [10]. The six key potential clinical applications of HPVDNA researches are: (i) triage of women with equivocal or low-grade cytological abnormalities; (ii) follow-up of

women with irregular screening results who are negative at colposcopy/biopsy; (iii) therapeutic outcome prediction after treatment of cervical intraepithelial neoplasia (CIN); (iv) Screening for *HPV* DNA mointoring, alone or in combination with a Pap smear, for detection of cervical-cancer precursors [11]; (v) Obtain useful information on the presence of other *HPV* forms; and (vi) National and country-based prevalence of type-specific *HPV* investigations, to provide baseline values for potential evaluation of the global impact of HPV vaccination(12). Morphological, serological and clinical findings can interfere with the existence of HPV. However, the diagnosis of *HPV* based on molecular-biology techniques which allow its accurate detection and typing. Nucleic acid hybridization tests, signal amplification tests and nucleic-acid amplification are currently underway [12], [13].

In the present study, the prevalence of HPV in cancer cervix patients by pathology and real time PCR were 15% and 14% respectively. Our findings are in close conformity with the data given by a multicentre case–control study and *human papillomavirus (HPV)* DNA prevalence was done in 15 countries and found that the prevalence was 13.4% in Brazil, Morocco, Paraguay, the Philippines, Thailand, Peru, Mali, Spain, and Colombia. 15.4% in Brazil, Morocco, Paraguay, the Philippines, Thailand, and Peru [14]. Also our results agree with a published work in Egypt at 2014 that reported *HPV* DNA in 10.4% of women [15]. In another work that conducted in Egypt (2006) reported the prevalence of *HPV* DNA in 15.06% of cases [16].

Spain reported 23 % prevalence rate [17].

In another published work, the results disagree with our work and found HPV DNA found in 93% of the cancer cervix in Colombia and Spain that can be explained that the authors test more than 20 HPV types as16,18,31,39,45,51,56 and 59 but in our study, we tested against two types only, and Possibly high prevalence of *HPV* among women due to high exposure of human immunodeficiency virus (HIV) in these countries [18]. Also, the prevalence of *HPV* in Qatar among the general population of female with normal or abnormal cytology has recently been estimated 6.1%. The authors reported the presence of different *HPV* types with a high prevalence of low-risk HPV types, especially type 81 [19].

The present study reported that the infection was single in 9% and mixed in 5% and HPV type 16 was higher than type 18 and these results were in agreement with a previous paper published at Egypt [15], 6.5% were infected with a single HPV type and 3.8% with mixed types and the most prevalent type was HPV 16. In another global work reported HPV DNA was higher than other types, the data was collected from 38 countries in Europe, North America, central South America, Africa, Asia, and Oceania [20] and also again is consistent with another global study [12].

Our study showed that there are two case were negative cases by real time PCR and positive by pathology in which HPV DNA was initially undetected because of specimen inadequacy, a relative predominance of integrated HPV-18, or technological inadequacy [21].

The age range across the sample was 18–55 years with a median age of 35 years of the women patient with subtype 18 had the highest median age (Md=48.5) across all subtypes and negative cases. And the distribution of age group and HPV infection found the highest level lies in age above 50 years and this was agree with another published work in Qatar and found a high prevalence of *HPV* infection in age above 55 years [22] and also again is consistent with another work published in Egypt that found The prevalence HPV type was highest (9.2%, 8/87) in the 45–54 years age group [15].

Association of PCR results and contraception use reveled a statistically significant difference and 6 cases were using OC. Evidence for an cervical cancer association of with the use of oral or other hormonal contraceptives is not entirely consistent. The use of OCs for five or more years is a cofactor that increases up to fourfold the risk of cervical cancer among women who are carriers of *HPV* DNA. These results provide a consistent summary of the previous publications of the individual studies included in the multicentre project [23]

Several researchers that study *HPV* positive female observed no associations or only weak associations in subgroup analyses [24]. Our results were agree with a previous published global paper included 1768 cases and 262 controls that were positive for *HPV* DNA. OC use "ever" was associated with a significant increase in risk[25].

CONCLUSION

Cervical cancer is the most fourth prevalent cancer that affects women worldwide, HPV is very important as a causative agent for cervical cancer, HPV 16 and 18 were the most prominent high risk HPV.

Recommendation:

More studies are needed with a more number of patients for more accurate results.

Conflicts of interest:

The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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