Cervical Carcinoma in Shatby University Hospital and its Relation to Human Papilloma Virus

Original Article

Hossam H. El Sokkary[1] and Reham Abo Elwafa[2]

[1]Department of Obstetrics and Gynecology, [2]Department of Clinical and Chemical Pathology, Faculty of Medicine, Alexandria University, Egypt

ABSTRACT

Objective: To detect human papilloma virus infections and its different genotypes in cervical cancer cases in Shatby obstetrics and gynecology university hospital of Alexandria medical school.

Patients and Methods: An observational analytical prospective cross-sectional study was managed on 70 cervical cancer patients collected from gyne-oncology unit in Shatby obstetrics and gynecology university hospital of Alexandria medical school. Collection of the sample from all cases of the study for human papilloma virus testing and genotyping was done.

Result: The study was conducted on 70 cervical cancer cases, considering human papilloma virus testing of the cases, the following results were found, 21 cases out of 70 cases (30%) were negative while 49 cases (70%) were positive. Collectively, 45 cases (64.3%) of the study cervical cancer cases were infected by high-risk HPV types (16,18,31), 4 cases (5,7%) by low-risk HPV types (6,11) and 21 cases (30%) were not infected.

Conclusion: The prevalence of high risk human papilloma virus positive cervical cancer cases in al Shatby Alexandria medical school university hospital was 64.3% and 55.4% of all the present study cervical cancer cases were infected by HPV types 16 and 18, the study assured the relationship between cervical cancer and high risk HPV especially types 16 and 18 and denoting the importance of HPV vaccination during adolescence and HPV testing as a screening test to detect early preinvasive cervical lesion.

Key Words: Cervical cancer, human papilloma virus, type 16 and 18 HPV.

Received: 23 October 2022, **Accepted:** 14 November 2022

Corresponding Author: Hossam Hassan Aly Hassan El Sokkary, MD, Department of Obstetrics and Gynecology, Faculty of Medicine, Alexandria University, Egypt, **Tel.:** +20 12 2385 0511, **E-mail:** hossamsokkary@hotmail.com

ISSN: 2090-7265, February 2023, Vol.13, No. 1

INTRODUCTION

Globally, cervical cancer is the third most common genital tract cancer after endometrial and ovarian and represents one of the important health problem worlds widely especially in developing countries[1,2]. Regarding mortality rate, cervical cancer is the second leading cause of death after ovarian cancer globally[3]. Considering Egypt, cervical cancer is the 11th most frequent female malignant disease aged between 15 to 45 years old[4]. Considering mortality rates in Egypt from cervical carcinoma, the estimates state that it ranks the 12th among the most common cause of death in Egyptian women[5]. For example in 2018, there were about 969 new cervical cancer cases in Egypt with 631 mortality cases from the same disease which constitutes a major health problem[6]. High risk genotypes of human papilloma virus represent one of the most important known cause of cervical cancer[7,8]. The early protein 6 and 7 (E6,E7) of high risk genotypes as types 16,18,31,33,etc work as an oncogenic protein[9]. E6 protein act by degrading P53 protein which is a tumor suppressor protein, this action reduces the half-life of p53 from several hours to less than 20 minutes[10,11]. E7 protein induces its oncogenic properties through suppression of retinoblastoma protein which inhibit E2F transcription factors by competing with E2F transcription factors for retinoblastoma protein so the net result is increasing free E2F transcription factors which transfers the division cycle of the cell to the next phase[12]. Knowing the incidence of cervical cancer cases in Egypt and in local community with high risk or low risk human papilloma virus genotypes infection is of great importance in prevention of this life threatening female cancer that represent an important health problem in Egypt and in local community by scheduling human papilloma virus vaccine to be one of the compulsory vaccine in adolescents female and by regular routine screening for early intraepithelial cervical lesion and early invasive cervical cancer through Papanicolaou smear test or liquid based cytology and human papilloma virus testing[13]. Relation of cervical cancer with other parameters as age, gravidity, parity, contraceptive history, medical diseases, pathological types, grades and disease stages in addition to frequency of human papilloma virus infection with different genotypes can give important knowledge about prevention, screening and management.

DOI:10.21608/ebwhj.2023.170507.1219

OBJECTIVE

To detect human papilloma virus infections and its different genotypes in cervical cancer cases in Shatby obstetrics and gynecology university hospital of Alexandria medical school.

PATIENTS AND METHODS

An observational analytical prospective cross-sectional study was managed from July 2019 to July 2022 on 70 cervical cancer patients collected from gyne-oncology unit in Shatby obstetrics and gynecology university hospital of Alexandria medical school after taking a written consent and following approval by Alexandria medical school institutional ethics committee. Inclusion criteria included cervical cancer patients diagnosed by histopathological examination after biopsy taking through colposcopic guided biopsy in suspicious cervix cases with abnormal Papanicolaou Smear (PAP smear) analyzed with Bethesda system, wedge and punch biopsy from exophytic cervical mass and endocervical curettage in unsatisfactory colposcopic examination in abnormal PAP smear or in endophytic lesion with barrel shaped cervix. Exclusion criteria included patients with large enough exophytic mass hiding transformation zone which is the site of cervical smear taking for human papilloma virus testing, patients with friable necrotic mass that shows bleeding on touch that lead to unproper cervical smear sampling due to its contamination with blood , pregnant patients, patients with cervical cone biopsy(no transformation zone), menstruating patients, or patients with abnormal uterine bleeding at the time of cytology sample collection, vaginal douching 2 days before sample tacking and patients using vaginal local contraceptive cream 48 hours before sample collection. All patients were subjected to full history taking as age, gravidity, parity, contraceptive history, medical disease, detailed marital history and smoking. Collection of the sample from all cases of the study for human papilloma virus testing and genotyping was done after diagnostic workup and before treatment of the study cases at any time in post-menopausal women and at mid cycles period in premenopausal women by cervical smear using PAP spatula and endocervical brush. Brush was used first, it was inserted and rotated half a circle inside endocervical canal then inserted inside Preservative Fluid contained a watery solution of small amounts of methanol, isopropanol and denatured ethanol followed by scraping of the cervical transformation zone using plastic PAP spatula which was immersed after into the preservative media. Great care was done during cervical smear taking to not touch malignant cervical exophytic masses to prevent bleeding and sample contamination with blood. The specimens were sent inside its preservative to laboratory within 24 hours after collection and stored at room temperature or at refrigerator with temperature (2-8ºC)[14]. Real time polymerase chain reaction using primers of high risk genotypes as 16, 18, 31, 33, 35, 39, 45 in addition to primers of low risk genotypes as 6 and 11 while genotyping was done through using fluorescein -labeled target specific probe which is formed of a nucleotide contain a reporter fluorescent dye and a quencher dye attached. If the target DNA sequence of the specific genotype is found after amplification by PCR, the probe anneals with one of the primers with cleavage of quencher dye attached and this led to increasing reporter signal that leads to increasing sensitivity of genotypes detection[15,16]. Cases were subdivided into 3 groups; group I HPV negative cases, group II low risk HPV positive cases and group III high risk HPV positive cases which included cases with mixed high risk and low risk HPV positive cases

Statistical Analysis

All data were collected, coded, tabulated, and statistically analyzed of the 3 groups using IBM SPSS statistics (Statistical Package for Social Sciences) software version 24.0, IBM Corp., Chicago, USA. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test. Quantitative data were described using mean and standard deviation for normally distributed data. For normally distributed data, comparison between more than two population were analyzed F-test (ANOVA) to be used. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level. The level of significance was taken at $P\ value < 0.05$ is statistically significant, otherwise is non-significant. The *p-value* is a statistical measure for the probability that the results observed in a study could have occurred by chance.

Justification of sample size

Sample size was estimated relied on a previous study and by using Med Calc statistical software. Assuming area under ROC to be 0.80, an alpha of 0.05 and power of study 90.0%, the beta error was 0.1. Typical advice is to reject the null hypothesis H0 if the corresponding *p-value* smaller than 0.05. a minimum sample size required was 70 patients will be required for this study.

RESULT

The study was conducted on 70 cervical cancer cases, considering human papilloma virus testing of the cases by real time PCR, the following results were found, 21 cases out of 70 cases (30%) were negative (group I) while 49 cases (70%) were positive. 39 cases(55.7%) out of 49 positive cases showed infection with high risk types of human papilloma virus distributed as follows; 23 cases (32.8%) were infected by type 16, 9 cases (12.8%) were infected by type 18 and 7 cases (10%) were infected by type 31 in comparison to 4 cases (5.7%) (group II) were infected by low risk types distributed as follows; 3 cases (4.2%) were infected by type 6 and 1 case (1.4%) was

infected by type 11. 6 cases (8.5%) out of 49 positive cases showed mixed infection with different genotypes, 4 (5.7%) of them were infected by 2 high risk types 16 and 18, 2 cases (2.8%)were infected by low and high risk types as follows; 1 (1.4%) with type 6 and 18 and the remaining case (1.4%) with 6,16,18.

Collectively, 45 cases (64.3%) (group III) of the study cervical cancer cases were infected by high risk HPV types (16,18,31), 4 cases (5,7%) by low risk HPV types (6,11) and 21 cases (30%) were not infected denoting a statistical significance difference between high risk HPV positive cervical cancer cases (group III) and the other 2 groups HPV negative cervical cancer cases (group I) and low risk HPV positive cervical cancer cases(group II) (p= 0.003) (Table 1, Figure 1).

Regarding demographic data of the study cases, a comparison was done between HPV negative cases (group I), high risk HPV positive cases (group III) and low risk HPV positive cases (group II). Considering age in HPV negative cases, it ranged from 38 to 75 years with mean 61.9 years, in low-risk HPV positive cases, it ranged from 44 to 60 years with mean 55.5 years in comparison to 24 to 72 years with mean 49.9 years in high-risk HPV positive cases. There was a significance difference between age in HPV negative cervical cancer cases and cases with high-risk HPV positive cases (p=0.023) as the mean age were higher in cases with negative HPV while there is no significant difference in mean age between cases with HPV negative and low risk HPV positive cases (p= 0.382) or cases with low risk and high-risk positive cases(p=0.115) (Table 2).

Considering residency in non-infected HPV cases, 17 (18%) out of 21 cases were from rural area and 4 (19%) out of 21 were from urban area, all the 4 cases of low risk were from rural area, in comparison to 9 cases (20%) out of 45 cases of the high-risk positive were from rural and 36 cases (80%) were from urban. There was a statistical significance difference between non HPV infected cases of cervical cancer and high risk positive cervical cancer cases as cases with high risk HPV infection showed higher percentage in urban area than in rural area and the reverse was true(p=0.003), the same was present between low risk and high risk HPV positive cervical cancer cases(p=0.001) and there was no significance difference considering residency between cases with no HPV infection and cases with cases with low risk HPV positive (p=0.284) (Table 2). Regarding smoking habit.

There was a statistical significance difference between non HPV infected cases of cervical cancer and high risk HPV types positive cervical cancer cases as cases with high risk infection showed higher percentage of smoking habit than non-smoker cases (p=0.031), the same was present between low risk and high risk HPV types positive cervical cancer cases (p=0.031) and there was no significance difference considering smoking habit between cases with no HPV infection and cases with cases with low risk HPV types positive (p=1) (Table 2). Considering gravidity and parity, There was a statistical significance difference between non HPV infected cases of cervical cancer and high risk HPV types positive cervical cancer cases as cases with high risk infection showed less gravidity and parity than cases with no HPV infection as p=0.001, p=0.014 respectively (Table 3) while there was a statistical significance between low risk and high risk HPV cases in relation to gravidity (p=0.019), this is not the case between same groups considering parity (p=0.158) (Table 3, Figure 2).

Again, there is no significance difference between non infected HPV cases and low risk HPV positive cases in relation to gravidity and parity (p=0.380, p=0.500) respectively (Table 3, Figure 2). Regarding contraceptive history, there was a significance difference between non HPV infected cases of cervical cancer and high risk HPV types positive cervical cancer cases, as cases with high risk infection showed more using of contraception whether intra uterine devices or oral contraceptive than non HPV infected cases (p=0.001), the same significance difference was present between low risk HPV positive and high risk HPV positive types with more contraception using with high risk HPV types (p=0.001) (Table 3). Regarding medical disease history, there was no significant difference between non infected HPV cases, low risk HPV positive cases and high-risk positive HPV cases (p1=0.059, p2= 0.071, p3=0.349) respectively as shown in table 3.

Regarding clinicoradiological staging and distribution of the cervical cancer cases in relation to non-infection with HPV , low risk HPV positive cases and high risk HPV positive cases were shown in table 4, considering surgical resectability, there was a significant difference between non HPV infected cases and high risk HPV positive types as non HPV infected cases were 100% not resectable advanced stage in comparison to 48.8 % of high risk HPV types positive cases were not resectable (p=0.02), while there was no significant difference between non infected HPV cases and low risk HPV positive types (p=0.103) or between low risk HPV types positive and high risk HPV types positive cases considering the same matter (p=0.032) as shown in (Table 4, Figure 3). Regarding pathological types and grading in relation to HPV infection, table 5 and figure 4 showed distribution of cases in relation to non-infection with HPV, low risk HPV positive cases and high-risk HPV positive cases.

Considering grading and different pathological types in relation to non-infection with HPV, low risk HPV positive cases and high risk HPV positive cases, there was a statistical difference between non infected cases and cases with high risk HPV positive types or low risk HPV positive types regarding adenocarcinoma grade 3 or undifferentiated

carcinoma as both types were associated with non-infected HPV cases than in high risk HPV positive types cases or low risk HPV positive types cases (p=0.021),(p=0.026) respectively while in relation to Non keratinizing large cell grade 2 or grade 3 were associated with high risk HPV positive types cases than the other 2 groups (p=0.033), (p=0.041) respectively as shown in (Table 5, Figure 4).

Table 1: Distribution of study cervical cancer cases according to Human papilloma virus infection and genotyping.

Human papilloma virus infection and risk types	Human papilloma virus genotyping	Number	Percentage	Total percentage	*P value*
HPV negative cases	-	21	30.0	30%	
Low risk HPV cases	6	5	7.1	5.7%	0.003*
	11	1	1.4		
High risk HPV cases	16	28	40.0	64.3%*	
	18	15	21.4		
	31	7	10.0		

Table 2: Comparison between study cervical cancer cases regarding Human papilloma virus infection, genotyping and basic demographic data.

	Group I "HPV negative cases" "n=21"		Group II "low risk HPV cases" "n=4"		Group III "High risk HPV cases" "n=45"		Test *P value*	P1 P2 P3
Age								
Range	38-75		44-60		24-72		ANOVa	0.382
Mean	61.95		55.5		49.09		8.32	*0.023
SD	9.51		7.72		10.05		0.039*	0.115
	No	%	No	%	No	%		
Residency								0.284
Rural	17	81.0	4	100.0	9	20.00	$X^{2=}$ 24.94	0.003*
Urban	4	19.0	0	0.0	36	80.00	0.001*	0.001*
Smoking habit								1.00
No	21	100.0	4	100.0	25	55.6	$X^{2=}$ 8.08	*0.031
Yes	0	0.0	0	0.0	20	44.4	0.017*	0.031*

Group I: HPV negative cases — Group II: low risk HPV positive cases — Group III: high risk HPV positive cases
ANOVA = ANOVA test — *P* was significant if < 0.05 — * Significant difference
P1 comparison between group I and II — P2 comparison between group I and III — P3 comparison between group II and III

Table 3: Comparison between study cervical cancer cases regarding Human papilloma virus infection and genotyping in relation to gravidity, parity, contraceptive history and medical disease.

	Group I "HPV negative cases" "n=21"		Group II "Low risk HPV cases" "n=4"		Group III "High risk HPV cases" "n=45"		Test *P value*	P1 P2 P3
Gravidity								
Range	2-11		4-7		0-12			0.380
Mean	5.19		5.5		3.89		Anova 16.25	0.001*
SD	1.94		1.29		2.60		0.003*	0.019*
Parity								
Range	2-9		4-7		0-8			0.500
Mean	4.76		5.0		3.07		Anova 9.25	0.014*
SD	1.41		1.41		1.75		0.041*	0.158
	No	%	No	%	No	%		
Contraceptive history								
None	1	4.8	0	0.0	8	17.78	$X^{2=}$	0.181
IUDS	19	90.5	4	100.0	11	24.44	26.70	0.001*
OCP	1	4.8	0	0.0	26	57.78	0.001*	0.001*
Medical disease								
None	11	52.4	1	25.0	27	60.00	$X^{2=}$	0.059
Hypertensive	2	9.5	0	0.0	3	6.67	2.851	0.071
Diabetic	8	38.1	3	75.0	15	33.33	0.582	0.349

Group I: HPV negative cases — Group II: low risk HPV positive cases — Group III: high risk HPV positive cases
ANOVA = ANOVA test — *P* was significant if < 0.05 — * Significant difference
P1 comparison between group I and II — P2 comparison between group I and III — P3 comparison between group II and III

Table 4: Comparison between study cervical cancer cases regarding Human papilloma virus infection and genotyping in relation to Clinico-radiological staging and resectability.

	Group I "HPV negative cases" "n=21"		Group II "Low risk HPV cases" "n=4"		Group III "High risk HPV cases" "n=45"		Test *P value*	P1 P2 P3
	No	%	No	%	No	%		
Clinoco-radiological staging								
3a	2	9.5	0	0	1	2.2		
4a	0	0	1	25	1	2.2		
1b1	0	0	0	0	5	11.1		
1b2	1	4.8	1	25	17	37.8	2.51	0.321
1b3	3	14.3	0	0	4	8.9	0.211	0.277 0.165
2b	14	66.7	2	50	15	33.3		
3b	1	4.8	0	0	1	2.2		
3c 1	0	0	0	0	1	2.2		
resectability								0.103
Non resectable	21	100.0	3	75.0	23	51.11	8.863	0.02*
Resectable	0	0.0	1	25.0	22	48.89	0.042*	0.032

Group I: HPV negative cases — Group II: low risk HPV positive cases — Group III: high risk HPV positive cases
ANOVA = ANOVA test — *P* was significant if < 0.05 — * Significant difference
P1 comparison between group I and II — P2 comparison between group I and III — P3 comparison between group II and III

Table 5: Comparison between study cervical cancer cases regarding Human papilloma virus infection and genotyping in relation to Pathological diagnosis.

Pathological diagnosis	Group I "HPV negative cases" "n=21"		Group II "Low risk HPV cases" "n=4"		Group III "High risk HPV cases" "n=45"		*P value*
	No	%	No	%	No	%	
Adenocarcinoma grade 3	6	28.57	0	0.0	3	6.7	0.021*
Non keratinizing large cell SSC grade 2	4	19.05	3	75.0	29	64.4	0.033*
Undifferentiated carcinoma with focal neuroendocrine differentiation	1	4.76	0	0.0	0	0.0	-
Non keratinizing large cell SSC grade 3	4	19.05	0	0.0	13	28.9	0.041*
Undifferentiated carcinoma	5	23.81	1	25.0	0	0.0	0.026*
Squamous cell carcinoma papillary variant grade 1	1	4.76	0	0.0	0	0.0	-

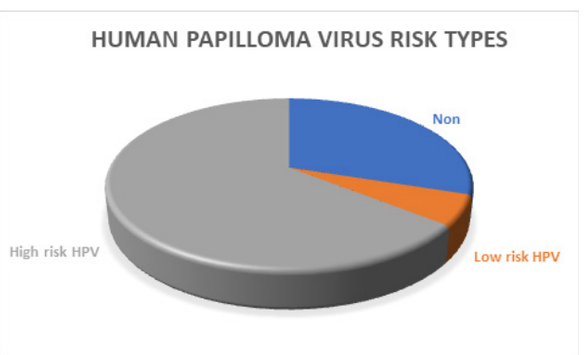

Fig. 1: Distribution of study cervical cancer cases according to Human papilloma virus infection and genotyping.

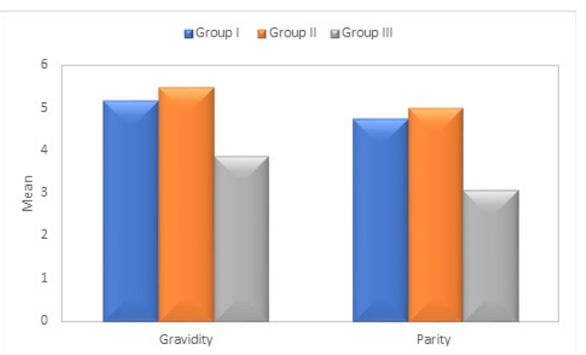

Fig. 2: Comparison between study cervical cancer cases regarding Human papilloma virus infection and genotyping in relation to gravidity and parity.
Group I: HPV negative cases
Group II: low risk HPV positive cases
Group III: high risk HPV positive cases

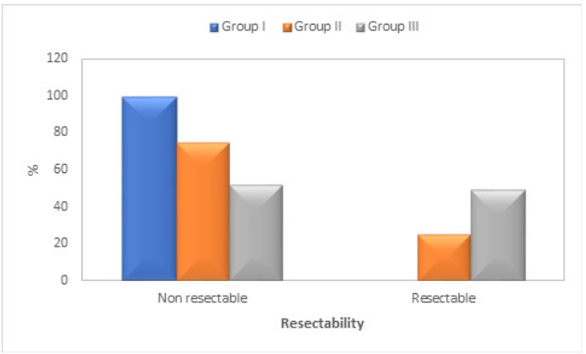

Fig. 3: Comparison between study cervical cancer cases regarding Human papilloma virus infection and genotyping in relation to resectability.
Group I: HPV negative cases
Group II: low risk HPV positive cases
Group III: high risk HPV positive cases

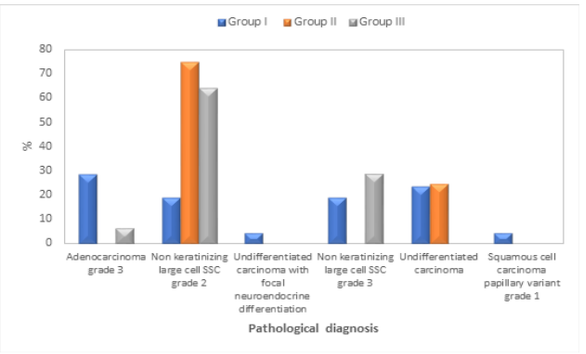

Fig. 4: Comparison between study cervical cancer cases regarding Human papilloma virus infection and genotyping in relation to Pathological diagnosis. Group I: HPV negative cases
Group II: low risk HPV positive cases
Group III: high risk HPV positive cases

DISCUSSION

Cervical cancer is a major health problem, in united states, every year about 14100 cases were diagnosed and 4280 cervical cancer cases were died[17] In developing countries the image is more worse, in 2018 a worldwide ranking considering the 50 higher incidence countries of cervical cancer was estimated and showed that the first 38 countries in this ranking were from Africa[18] In Egypt, the incidence is 2.3 cases for every 100000 women every year which constitutes a great concerning situation in country that population exceed 100 million[19].

The relation between high risk types of HPV and pathogenesis of cervical cancer was well known through E6 and E7 viral protein[20] In this article we try to identify this relationship between cervical cancer and HPV in our local community by estimation human papilloma virus infection rates and different genotypes in cervical cancer cases of El Shatby university Alexandria medical school hospital so we can estimate magnitude of the problem and the importance of HPV vaccination and HPV testing as an essential screening test with PAP smear as both procedure can be omitted due to cost issue. The study was enrolled on 70 cervical cancer cases and the result showed that 45 cases (64.3% of the study cases) were infected by high-risk genotypes, in comparison to 21 cases (30%) showed no infection and 4 cases (5.7%) with low-risk types. The result assured the importance of HPV vaccination and HPV contesting with PAP smear.

In agreement with the present study, de Sanjose S, Quint WG, Alemany L[21] et al investigated the infection of invasive cervical cancer with high risk HPV genotypes and found that about 85% of the cases showed high risk HPV infection and type 16 and 18 were present in 71% of the study cases in comparison to 65.4% of the present study cases were infected by high risk types as types 16, 18, 31 and types 16 and 18 were present in 55.4% of the study cases. The difference between both studies may be due to different HPV testing technique the present study used real time PCR on cervical smearing sample while de Sanjose S, Quint WG, Alemany L et al used PCR testing on paraffin embedded cervical cancer tissue to detect HPV genome which may have different sensitivity.

Abd El-Moneim et al found a comparable result to the present study and their results showed that 40% of the cases were infected by high risk HPV genotypes and types 16 and 18 were present in 30% of cervical cancer cases[22] The difference between the last study and the present one that all cases of Abd El-Moneim et al study were early stages or preclinical stages diagnosed by coloposcopic guided biopsy in contrast to the present study which included clinical stages and 47 out of 70 cases (67.1%) were unresectable.

Regarding demographic data, there was a significance difference between age in HPV negative cervical cancer cases and cases with high-risk HPV positive cases as the mean age were higher in cases with negative HPV, in agreement with the present study, Rosa Schulte-Frohlinde, Damien Georges et al found that incidence of cervical cancer was higher at early age in high-risk HPV infected cervical cancer patients than in non HPV infected cases[23]

In relation to residency There was a statistical significance difference between non-HPV infected cases of cervical cancer and high risk positive cervical cancer cases as cases with high-risk HPV infection showed higher percentage in urban area than in rural area, the last finding denoting the importance of HPV testing as screening methods of early cervical lesion in urban than in rural area in our local community. The last result was in controversy to other study as Zahnd, Whitney et al study that showed high incidence of HPV related genital tract cancer than in urban area[24] The explanation of last finding that the present study community showed lack in HPV vaccination and HPV testing in cervical cancer in both urban and rural area but because of high prevalence of high risk types of HPV in urban area due to multiple marriage resulted in high percentage of high risk HPV positive cervical cancer cases in urban area than in rural area.

From the previous data, the present study showed the importance of HPV vaccination in young female adolescents especially in urban area in study local community and to offer a screening program to all married women that should begin from 21 years old or after marriage whatever early to prevent a massive lethal health problem.

CONCLUSION

The prevalence of high-risk human papilloma virus positive cervical cancer cases in al shatby Alexandria medical school university hospital was 64.3% and types 16 and 18 HPV genotypes infected cases constituted 55.4% of all the present study cervical cancer cases. The study assured the relationship between cervical cancer and high-risk HPV especially types 16 and 18 and denoting the importance of HPV vaccination during adolescence and HPV testing as a screening test to detect early preinvasive cervical lesion.

RECOMMENDATIONS

Multi randomized further studies are needed in different local community in Egypt to detect prevalence of high-risk HPV types in preclinical and clinical cervical cancer lesion so we can precisely estimate the importance of HPV vaccination and HPV testing as a screening test for preclinical cervical neoplastic lesions.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Lindsey A. Torre, Farhad Islami, Rebecca L. Siegel, Elizabeth M. Ward, Ahmedin Jemal; Global Cancer in Women: Burden and Trends. Cancer Epidemiol Biomarkers Prev 1 April 2017; 26 (4): 444–457. https://doi.org/10.1158/1055-9965.EPI-16-0858

2. Hull R, Mbele M, Makhafola T, Hicks C, Wang SM, Reis RM, Mehrotra R, Mkhize-Kwitshana Z, Kibiki G, Bates DO, Dlamini Z. Cervical cancer in low and middle-income countries. Oncol Lett. 2020 Sep;20(3):2058-2074. doi: 10.3892/ol.2020.11754. Epub 2020 Jun 19. PMID: 32782524; PMCID: PMC7400218.

3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021:71:209–49. doi:10.3322/caac.21660.

4. Bruni, L., Albero, G., Serrano, B., Mena, M., Collado, J. J., Gómez, D., *et al*. (2021). ICO/IARC information centre on HPV and cancer (HPV Information Centre). Human papillomavirus and related diseases in Egypt. Summary Report.

5. Arbyn, M., Weiderpass, E., Bruni, L., de Sanjosé, S., Saraiya, M., Ferlay, J., & Bray, F. (2020). Estimates of incidence and mortality of cervical cancer in 2018: A worldwide analysis. The Lancet Global Health, 8(2), e191–e203.

6. Ferlay, J., Ervik, M., Lam, F., Colombet, M., Mery, L., Piñeros, M., *et al*. (2018). Global cancer observatory: cancer today. Lyon, France: International Agency for Research on Cancer.

7. Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer. Lancet. 2013 Sep 7;382(9895):889-99. doi: 10.1016/S0140-6736(13)60022-7. Epub 2013 Apr 23. PMID: 23618600.

8. zur Hausen, H. (2009). Papillomaviruses in the causation of human cancers—a brief historical account. Virology, 384(2), 260–265.

9. Schmitt, A., J. B. Harry, B. Rapp, F. O. Wettstein, and T. Iftner. 1994. Comparison of the properties of the E6 and E7 genes of low- and high-risk cutaneous papillomaviruses reveals strongly transforming and high Rb-binding activity for the E7 protein of the low-risk human papillomavirus type 1. J. Virol. 68:7051-7059.

10. Hubbert, N. L., S. A. Sedman, and J. T. Schiller. 1992. Human papillomavirus type 16 E6 increases the degradation rate of p53 in human keratinocytes. J. Virol. 66:6237-6241.

11. Huibregtse, J. M., M. Scheffner, and P. M. Howley. 1991. A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. EMBO J. 10:4129-4135.

12. Fischer, M., Uxa, S., Stanko, C. *et al*. Human papilloma virus E7 oncoprotein abrogates the p53-p21-DREAM pathway. Sci Rep 7, 2603 (2017). https://doi.org/10.1038/s41598-017-02831-9

13. Moyer, VA; U.S. Preventive Services Task, Force (Jun 19, 2012). "Screening for cervical cancer: U.S. Preventive Services Task Force recommendation statement". Annals of Internal Medicine. 156 (12): 880–91, W312. doi:10.7326/0003-4819-156-12-201206190-00424

14. Lin CQ, Zeng X, Cui JF, Liao GD, Wu ZN, Gao QQ, Zhang X, Yu XZ, Chen W, Xi MR, Qiao YL. Stability Study of Cervical Specimens Collected by Swab and Stored Dry Followed by Human Papillomavirus DNA Detection Using the cobas 4800 Test. J Clin Microbiol. 2017 Feb;55(2):568-573. doi: 10.1128/JCM.02025-16. Epub 2016 Dec 7. Erratum in: J Clin Microbiol. 2017 Jun;55(6):1972. PMID: 27927922; PMCID: PMC5277527.

15. Heid C.A., Stevens J., Livak K.J., Williams P.M. Real time quantitative PCR. Genome Res. 1996; 6:986–994.

16. Bonetta L. Prime time for real-time PCR. Nat Methods. 2005; 2:305–312.

17. Fontham, ETH, Wolf, AMD, Church, TR, *et al*. Cervical Cancer Screening for Individuals at Average Risk: 2020 Guideline Update from the American Cancer Society. CA Cancer J Clin. 2020. https://doi.org/10.3322/caac.21628.

18. Stelzle D, Tanaka LF, Lee KK, Ibrahim Khalil A, Baussano I, Shah ASV, *et al*. Estimates of the global burden of cervical cancer associated with HIV. Lancet Glob Heal [Internet]. 2021;9(2):e161-9. Available from: https://doi.org/10.1016/S2214-109X(20)30459-9.

19. World Health Organization Information Centre on HPV and Cancer (2010) Human Papillomavirus and Related Cancers in Egypt. Summary Report. WHO/ICO.

20. Yim EK, Park JS. The role of HPV E6 and E7 oncoproteins in HPV-associated cervical carcinogenesis. Cancer Res Treat. 2005 Dec;37(6):319-24. doi: 10.4143/crt.2005.37.6.319. Epub 2005 Dec 31. PMID: 19956366; PMCID: PMC2785934.

21. De Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, Tous S, Felix A, Bravo LE, Shin HR, Vallejos CS, de Ruiz PA, Lima MA, Guimera N, Clavero O, Alejo M, Llombart-Bosch A, Cheng-Yang C, Tatti SA, Kasamatsu E, Iljazovic E, Odida M, Prado R, Seoud M, Grce M, Usubutun A, Jain A, Suarez GA, Lombardi LE, Banjo A, Menéndez C, Domingo EJ, Velasco J, Nessa A, Chichareon SC, Qiao YL, Lerma E, Garland SM, Sasagawa T, Ferrera A, Hammouda D, Mariani L, Pelayo A, Steiner I, Oliva E, Meijer CJ, Al-Jassar WF, Cruz E, Wright TC, Puras A, Llave CL, Tzardi M, Agorastos T, Garcia-Barriola V, Clavel C, Ordi J, Andújar M, Castellsagué X, Sánchez GI, Nowakowski AM, Bornstein J, Muñoz N, Bosch FX; Retrospective International Survey and HPV Time Trends Study Group. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. 2010 Nov;11(11):1048-56. doi: 10.1016/S1470-2045(10)70230-8. Epub 2010 Oct 15. PMID: 20952254.

22. Mohamed Ibrahim Ghorab, A., Abd El-Hady Mohamed, M., Mohamad Mohamad Eid, S., Mohamed Ahmed Saleh, M., El-Din Sayed Omar Semary, S. DETECTION OF HUMAN PAPILLOMAVIRUS GENOTYPES IN CANCER CERVIX PATIENTS: DAMIETTA GOVERNORATE, EGYPT. Al-Azhar Medical Journal, 2019; 48(4): 407-420. doi: 10.21608/amj.2019.64948

23. Rosa Schulte-Frohlinde, Damien Georges, Gary M Clifford, Iacopo Baussano, Predicting Cohort-Specific Cervical Cancer Incidence from Population-Based Surveys of Human Papilloma Virus Prevalence: A Worldwide Study, American Journal of Epidemiology, Volume 191, Issue 3, March 2022, Pages 402–412, https://doi.org/10.1093/aje/kwab254.

24. Zahnd, Whitney & Rodriguez, Christofer & Jenkins, Wiley. (2018). Rural-Urban Differences in Human Papillomavirus-associated Cancer Trends and Rates: Rural-Urban Differences in HPVa Cancer Trends. The Journal of Rural Health. 35. 10.1111/jrh.12305.