



CODEN [USA]: IAJ PBB

ISSN : 2349-7750

INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

<https://doi.org/10.5281/zenodo.5233325>Online at: <http://www.iajps.com>

Research Article

THE CORRELATION BETWEEN HUMAN PAPILLOMA VIRUS INFECTION AND ABNORMAL CERVICAL SMEARS IN SAUDI ARABIA

Fatimah Al-Mubarak*, Dana Al-Mishri, Fayez Al-Zahrani, Esra'a Al-Zaid, Batool Alali,
Mustafa Alsayhathi

King Fahad Specialist Hospital-Dammam, Ammar Bin Thabet Street, Dammam, Kingdom of
Saudi Arabia.

Article Received: July 2021

Accepted: July 2021

Published: August 2021

Abstract:

Background: The prevalence of human papillomavirus (HPV) infection is progressively increasing. HPV is responsible for 70% of cervical cancers worldwide; moreover, cervical cancer is the fourth leading cause of death in women.

Aim: This study investigated the association between abnormal cervical smears and HPV infection in Saudi Arabian women.

Methods: An analytical, descriptive, retrospective, patient-based cross-sectional study was conducted in Dammam, Saudi Arabia, from January to February 2020. The World Health Organization questionnaire was adopted and modified to suit Saudi culture. Patient files were reviewed, and phone interviews were conducted. Data were collected from 60 participants, with a response rate of 70%.

Results: A total of 51.7% HPV-positive samples were detected, and the majority were found in women aged over 40 years, multiparas, and in those who had their first sexual encounter at age 12–23 years. There was no significant association between the use of any form of hormonal contraceptive by either the women or their partners and the risk of HPV or cervical abnormality.

Conclusion: The prevalence of HPV infection was high among the women with abnormal cervical biopsies. The end-result of cervical cancer emphasizes the importance of national screening programs and inclusion of the HPV vaccine in the national immunization program.

Key words: cervical cancer, human papillomavirus, primary health care, screening, abnormal Pap smear

Corresponding author:**Dr. Fatimah Al-Mubarak,**

Family Medicine Department, King Fahad Specialist Hospital-Dammam
Ammar Bin Thabet Street, Dammam 15215/31444, Kingdom of Saudi Arabia.

Tel: +966565483233

Email: Fzm_mub@hotmail.com

QR code



Please cite this article in press Fatimah Al-Mubarak et al., "The Correlation Between Human Papilloma Virus Infection And Abnormal Cervical Smears In Saudi Arabia", *Indo Am. J. P. Sci.* 2021; 08(08).

INTRODUCTION:

The prevalence of human papillomavirus (HPV) infection has increased progressively among women throughout the world [1] It is responsible for 70% of cervical cancers worldwide; moreover, cervical cancer is the fourth leading cause of death from cancer in women. In Saudi Arabia, cervical cancer is the eleventh most common cancer among women [1]

Evidence linking HPV to cervical cancer is extensive; all cases of cervical cancer are attributable to HPV infection, with HPV-16 accounting for approximately 50% of cases and HPV-18 for 20% of cases. The risk of HPV infection in both sexes is related to the number of sexual partners. Further, both vaginal and anal intercourse are major risk factors for HPV infection [2]. There is a strong relationship between cervical abnormalities, including cervical cancer, and HPV infection; however, upon reviewing the literature, data regarding HPV infection prevalence and its association with any type of cervical abnormality was found to be minimal in Saudi Arabia. A recent study conducted in 2015 in Saudi Arabia states that “despite tremendous efforts, information on HPV prevalence in developing countries generally remains insufficient, and data on the HPV burden in Saudi Arabia are not yet available.”[1].¹ Another study published in 2014 on women residing in Riyadh, Saudi Arabia determined HPV prevalence in cervical specimens and revealed that of 519 cervical specimens, 31.6% were positive for HPV and that a significant association existed between HPV positivity and abnormal cytology [3]. Another study conducted at King Abdulaziz University Hospital (KAUH) in Jeddah on Saudi women with cervical cancer found HPV infection in 43% of 40 cervical specimens, and the prevalence was highest among those aged 35–76 years and decreased slowly in the older age groups. Consequently, the authors recommended that the significance of HPV prevalence in an area should be considered for vaccination programs [4].

Several biomarker-based methods, including molecular methods and immunohistochemical staining, have been used to detect high-risk HPV types in cervical lesions. Overexpression of p16 protein, a well-known surrogate marker for high-risk HPV-associated lesions, can be detected by immunohistochemical staining. According to a study by Gothwal M *et al.*, p16 immunostaining is suggestive of true HPV infection of the cells [5]. Further, to improve diagnostic accuracy, other markers, including Ki-67, have been used in conjunction with p16 in the histological assessment of cervical lesions. Similar to p16, Ki-67 is

overexpressed in high-grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma. In seven studies comprising 2628 patients, the pooled sensitivity and specificity of p16 conjugated with Ki-67 (both used in our study) for triage of abnormal Papanicolaou (Pap) smear results were 0.91 (95% CI, 0.89 to 0.93) and 0.64 (95% CI, 0.62 to 0.66), respectively [6]. Our study aimed to assess the correlation between the prevalence of HPV infection and abnormal cervical smears among women in Saudi Arabia.

MATERIAL AND METHODS:

An analytical, descriptive, retrospective, patient-based cross-sectional study was conducted. All Saudi and non-Saudi women with abnormal Pap smear results during the last 10 years, who were also tested for HPV infection were included in the study. HPV testing was done at King Fahad Specialist Hospital-Dammam by p16 and Ki-67 immunostaining of cervical biopsies that were performed routinely after an abnormal cervical smear. Participants aged below 21 years and above 65 years, men, deceased, and those unable to communicate were excluded. A World Health Organization (WHO) questionnaire—the International Agency for Research on Cancer (IARC) HPV prevalence survey—was adopted and modified to suit Saudi culture. The questionnaire was translated by an authorized translation office, reviewed by consultants from different specialties for validation, and labeled with a serial number to conceal the identity of the women. A data collection sheet was prepared using the adopted questionnaire variables, including HPV status, and was filled by reviewing medical records of the women along with conducting phone interviews; further, verbal consent was obtained from all participants before gathering information. Data were entered into a personal computer and analyzed using the Statistical Package for Social Sciences (SPSS). Data were coded, checked for completeness, sorted, and verified to eliminate inconsistencies or outliers. Data were then computerized, processed, and analyzed. Descriptive statistics were presented as numbers and percentages (%). The relationship between HPV infection and different characteristics of the women was assessed using the chi-square test. Variables that were significant were placed in the multivariate regression model to determine the independent significant predictor of HPV positivity; further, the odds ratio and 95% confidence interval were reported. A two-sided p-value <0.05 was used to indicate statistical significance. The study was approved by the Institute of Research Board (IRB) and the Ethical Committee of the King Fahad Specialist Hospital-Dammam prior to initiation of the study. The

Collaborative Institutional Training Initiative (CITI) and National Institute of Health (NIH) certifications were obtained by all authors. The privacy and confidentiality of the participating women were safeguarded throughout the study.

RESULTS:

A total of 60 women were recruited for this study, and Table 1 presents their socio-demographic

characteristics. More than half (51.7%) of the women were in the older group (>40 years), with nearly all being Saudis (96.7%) and 26.7% living in Qatif, Saudi Arabia. Regarding the educational level, 51.7% had secondary education or lower, and the rest (48.3%) had bachelor's or higher degrees. Furthermore, a majority of the women declared having been married only once, and 60% of them were married at age 19–25 years.

Table 1: Socio-demographic characteristics of the women (n=60)

Study data	N (%)
Age group in years	
• 24–40 years	29 (48.3%)
• >40 years	31 (51.7%)
Nationality	
• Saudi	58 (96.7%)
• Non-Saudi	02 (03.3%)
Residence location	
• Dammam	13 (21.7%)
• Qatif	16 (26.7%)
• Al Ahsa	10 (16.7%)
• Other	21 (35.0%)
Educational level	
• Secondary or lower	31 (51.7%)
• Bachelor or higher	29 (48.3%)
Occupational status	
• Unemployed	38 (63.3%)
• Employed	22 (36.7%)
Family monthly income (SAR)	
• <5,000	12 (20.0%)
• 5000–9999	18 (30.0%)
• ≥10,000	09 (15.0%)
• I do not want to share	21 (35.0%)
Marital status	
• Single	01 (01.7%)
• Married once	52 (86.7%)
• Married twice or more	03 (05.0%)
• Separated/Divorced	03 (05.0%)
• Widowed	01 (01.7%)
Age at marriage	
• Not married	01 (01.7%)
• ≤18 years old	16 (26.7%)
• 19–25 years old	36 (60.0%)
• >25 years old	07 (11.7%)
Smoking	
• Smoker/Ex-smoker	05 (08.3%)
• Non-smoker	55 (91.7%)

Details of the reproductive history of the women are discussed in Table 2. It shows that 45% of women reported having their first menstrual period at age 12–15 years. Likewise, 41.7% of them reported having been pregnant four to five times. Further, the proportion of women who stated that they or their partner used contraceptives was 75%, of which 25% reported having used hormonal contraceptives for less than a year.

Table 2: Reproductive history of the women (n=60)

Variables	N (%)
Age at first menstrual period	
• <9 years old	03 (05.0%)
• 9–11 years old	26 (43.3%)
• 12–15 years old	27 (45.0%)
• >15 years old	04 (06.7%)
Have you had menopause?	
• Yes	15 (25.0%)
• No	45 (75.0%)
If post-menopausal, at what age did you have your last menstrual period?	
• I have not gone through menopause	45 (75.0%)
• <40 years old	04 (06.7%)
• 40–45 years old	04 (06.7%)
• 46–50 years old	05 (08.3%)
• >50 years old	02 (03.3%)
How many times have you been pregnant?	
• Never been pregnant	06 (10.0%)
• One	06 (10.0%)
• 2–3 times	15 (25.0%)
• 4–5 times	25 (41.7%)
• >5 times	08 (13.3%)
How old were you when you had your first pregnancy?	
• Never been pregnant	06 (10.0%)
• Cannot remember	06 (10.0%)
• 14–22 years old	23 (38.3%)
• >22 years old	25 (41.7%)
Have you ever had a hysterectomy?	
• Yes	14 (23.3%)
• No	46 (76.7%)
Have you or your partner ever used contraceptives?	
• Yes	45 (75.0%)
• No	15 (25.0%)
How many years have you been taking any type of hormonal contraceptives?	
• Not used	25 (41.7%)
• <1 year	15 (25.0%)
• 1–5 years	10 (16.7%)
• >5 years	10 (16.7%)

Figure 1 shows the types of contraceptives used. The most used contraceptives were birth control pills (68.1%), followed by condoms (40.4%), and intrauterine devices (23.4%).

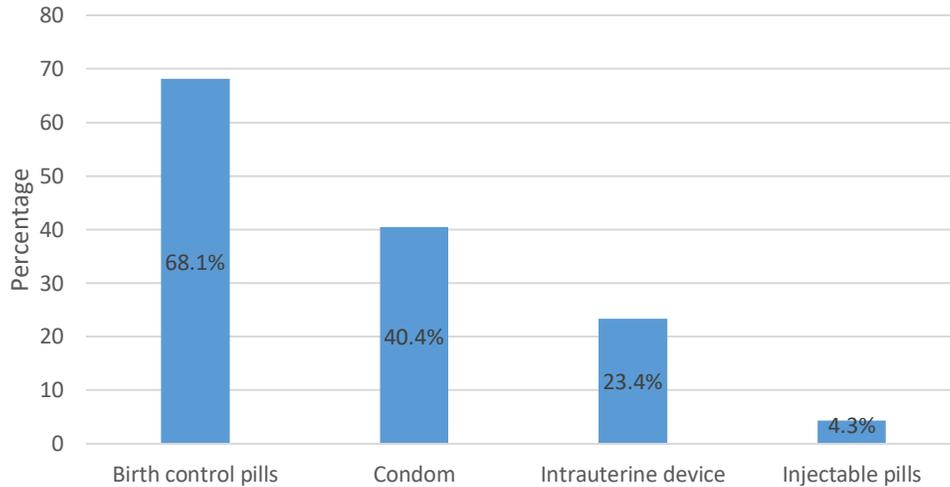


Figure 1: Types of contraceptives

Figure 2 depicts the prevalence of HPV infection, wherein 51.7% of women were HPV-positive, while the rest (48.3%) were HPV-negative.

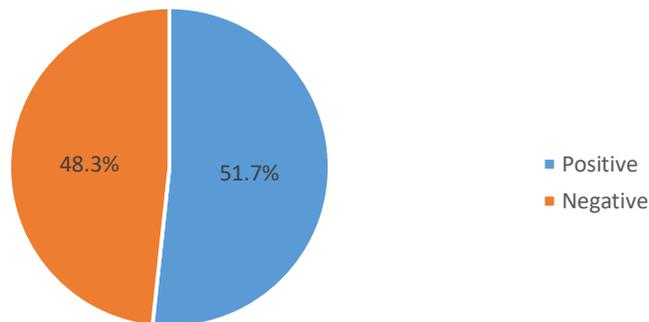


Figure 2: Prevalence of HPV infection

Figure 3 shows that the most common Pap smear findings were squamous cell carcinoma (30%), followed by cervical cancer (21.7%) and cervical intraepithelial neoplasia (CIN) (21.7%), while the least common was adenocarcinoma (11.7%).

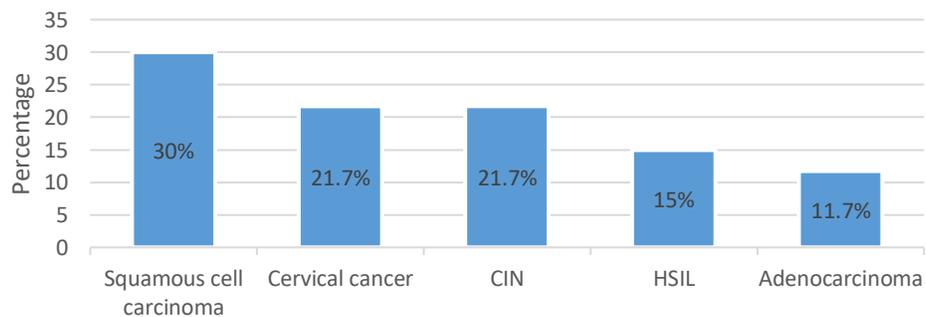


Figure 3: Most common Pap smear findings

Table 3 shows the Pap smear test history and sexual history of the women. According to the results, 43.3% of the women indicated having had their first sexual encounter at an age between 12 and 23 years, and most of them (63.3%) reported that they had sexual intercourse with their partner only throughout their life. Further, 15% of women declared that their current partner had had a sexual encounter with another person before starting to have sexual intercourse with them.

Table 3: Pap smear test history and sexual history of the women (n=60)

Variables	N (%)
Previous history of Pap test	
• Yes	50 (83.3%)
• No	10 (16.7%)
When did you have your last Pap test? (n=50)	
• <1 year ago	12 (24.0%)
• 1–5 years ago	16 (32.0%)
• >5 years ago	05 (10.0%)
• Cannot remember	17 (34.0%)
Age at first sexual encounter	
• No sexual encounter	17 (28.3%)
• 12–23 years old	26 (43.3%)
• 24–38 years old	17 (28.3%)
Throughout your life, how many different men have you had sexual intercourse with?	
• None	17 (28.3%)
• Only partner	38 (63.3%)
• More than one	05 (08.3%)
As far as you know, has your current partner/spouse ever had sex with another woman “before” you started having sex with him?	
• Yes	09 (15.0%)
• No	30 (50.0%)
• I do not know	21 (35.0%)

The chi-square test was used in Table 4 to evaluate the relationship between HPV and socio-demographic characteristics of the women. The results show that the prevalence of HPV positivity was significantly higher among women in the older age group (>40 years) ($X^2=4.241$; $p=0.039$).

Table 4: Relationship between HPV infection and socio-demographic characteristics of the women (n=60)

Factor	HPV		X ²	p-value [§]
	Positive N (%) (n=31)	Negative N (%) (n=29)		
Age group in years				
• 24–40 years	11 (35.5%)	18 (62.1%)	4.241	0.039**
• >40 years	20 (64.5%)	11 (37.9%)		
Residence location				
• Damman	08 (25.8%)	05 (17.2%)	2.076	0.557
• Qatif	06 (19.4%)	10 (34.5%)		
• Al Ahsa	06 (19.4%)	04 (13.8%)		
• Other	11 (35.5%)	10 (34.5%)		
Educational level				
• Secondary or lower	15 (48.4%)	16 (55.2%)	0.276	0.599
• Bachelor or higher	16 (51.6%)	13 (44.8%)		
Occupational status				
• Unemployed	17 (54.8%)	21 (72.4%)	1.993	0.158
• Employed	14 (45.2%)	08 (27.6%)		
Family monthly income (SAR)				
• <5,000	05 (26.3%)	07 (35.0%)	0.641	0.726
• 5,000–9,999	10 (52.6%)	08 (40.0%)		
• ≥10,000	04 (21.1%)	05 (25.0%)		
Age at marriage				
• ≤18 years old	12 (38.7%)	04 (14.3%)	5.258	0.072
• 19–25 years old	17 (54.8%)	19 (67.9%)		
• >25 years old	02 (06.5%)	05 (17.9%)		

[§] p-value was calculated using the chi-square test.

** Statistically significant at p<0.05 level.

We assessed the relationship between HPV and Pap smear findings, sexual history, and reproductive history of the women. The prevalence of HPV positivity was significantly higher among patients with a previous history of a Pap smear test ($X^2=4.819$; $p=0.028$), while the prevalence of HPV positivity was significantly less among those who had a sexual encounter at the age of 24–38 years ($X^2=9.627$; $p=0.008$) and those who had more than one sexual encounter with their partner ($X^2=6.590$; $p=0.037$) (Table 5).

Table 5: Relationship between HPV infection and Pap smear test history, sexual history, and reproductive history of the women (n=60)

Factor	HPV		X ²	p-value [§]
	Positive N (%) (n=31)	Negative N (%) (n=29)		
Age at first menstrual period				
• <12 years old	13 (41.9%)	16 (55.2%)	1.051	0.305
• ≥12 years old	18 (58.1%)	13 (44.8%)		
Have you had menopause?				
• Yes	08 (25.8%)	07 (24.1%)	0.022	0.881
• No	23 (74.2%)	22 (75.9%)		
How many times have you been pregnant?				
• ≤3 times	10 (35.7%)	11 (42.3%)	0.247	0.619
• >3 times	18 (64.3%)	15 (57.7%)		
Have you ever had a hysterectomy?				
• Yes	09 (29.0%)	05 (17.2%)	1.164	0.281
• No	22 (71.0%)	24 (82.8%)		
Have you or your partner ever used contraceptives?				
• Yes	24 (77.4%)	21 (72.4%)	0.200	0.655
• No	07 (22.6%)	08 (27.6%)		
Most recent Pap smear findings				
• Cervical cancer	07 (22.6%)	06 (20.7%)	2.324	0.676
• Squamous cell carcinoma	10 (32.3%)	08 (27.6%)		
• Adenocarcinoma	05 (16.1%)	02 (06.9%)		
• HSIL	04 (12.9%)	05 (17.2%)		
• CIN	05 (16.1%)	08 (27.6%)		
Previous history of Pap test				
• Yes	29 (93.5%)	21 (72.4%)	4.819	0.028**
• No	02 (06.5%)	08 (27.6%)		
Age at first sexual encounter				
• No sexual encounter	13 (41.9%)	04 (13.8%)	9.627	0.008**
• 12–23 years old	14 (45.2%)	12 (41.4%)		
• 24–38 years old	04 (12.9%)	13 (44.8%)		
Throughout your life, how many different men have you had sexual intercourse with?				
• None	13 (41.9%)	04 (13.8%)	6.590	0.037**
• Only partner	15 (48.4%)	23 (79.3%)		
• More than one	03 (09.7%)	02 (06.9%)		
As far as you know, has your current partner/spouse ever had sex with another woman “before” you started having sex with him?				
• Yes	04 (12.9%)	05 (17.2%)	2.914	0.233
• No	13 (41.9%)	17 (58.6%)		
• I do not know	14 (45.2%)	07 (24.1%)		

[§] p-value was calculated using the chi-square test.

** Significant at p<0.05 level.

DISCUSSION:

The role of high-risk HPV infection as an etiologic cause in the development of precancerous and cancerous cervical lesions has been well established. Understanding the HPV life cycle in an infected cell enables the identification of several biomarkers that can detect HPV presence in women who are at risk of developing cancer. Assays for HPV detection work to detect either HPV DNA, HPV RNA, and HPV proteins, mainly E6, or cellular biomarkers. Immunohistochemical staining, which we used in our study, detects cellular biomarkers such as p16 protein. Moreover, to improve diagnostic accuracy, we conjugated it with Ki-67 (cell proliferation marker); co-expression of p16 and Ki-67 has proven to predict the presence of high-risk HPV infection due to deregulation of the cell cycle [7]. In a prospective study (from October 2009 to November 2012), 1123 women were referred to colposcopy either due to positive HPV testing or abnormal cytology results (atypical squamous cells of unknown significance or worse abnormalities). Cytology specimens were used for HPV detection by DNA detection method and dual-staining with p16/Ki-67. The study concluded that the use of the molecular markers p16 and Ki-67 has higher specificity than DNA detection tests for HSIL or cervical cancer, and this may support the use of p16/Ki-67 dual-staining in the triage of patients referred for abnormal screening results [8].

Owing to the Saudi culture, disclosing information about sexual issues is a sensitive matter, which decreases the detection rate of HPV infection and delays the diagnosis of cervical cancer, leading to poor outcomes. This study identified 51.7% of women with positive HPV infection, and the majority (64.5%) were aged above 40 years. These findings are comparable with that of another study, conducted in the United States during 2013–2014, which showed an HPV prevalence of 39.9% among women aged 18–69 years [9].

In this study, early sexual activity was identified as one of the risk factors for HPV positivity based on the regression model ($p=0.016$); women who had their first sexual encounter at age 12–23 years had a higher risk of being HPV positive. This finding was supported by another pooled study that identified the same risk factor to be related to a high risk of HPV infection [10]. These results emphasize the importance of sexual education, which can be included in school health programs, among adolescents.

Regarding the association between the risk of HPV infection or cervical abnormality and use of

contraceptives, the proportion of women who stated that they or their partner had used some form of contraception was 75%, of which 68.1% used hormonal pills; moreover, 25% of them reported using hormonal contraceptives for less than a year and the remaining for 1 to >5 years, with no significant association. This was supported by a study published in May 2003 that stated that “there is no strong positive or negative relation between oral contraceptive use and prevalent infection of the cervix with HPV.” [11]. The results of this study suggest a high prevalence of HPV among women and that its relation to cervical abnormalities, including cervical cancer, are clinically significant to support the importance of screening and preventive measures, either by Pap smear or vaccination programs. Further, as multiparas are at high risk of HPV infection, good family planning and women’s health programs, which can be easily achieved in primary care visits, are warranted.

Limitation of the study

The sample size was small, and only 60 participants were included. The number of samples was limited due to the lack of a specific HPV screening tool; there are no national screening programs for HPV, and not all hospitals have access to a validated tool. Our center is an oncology center, and it uses immunostaining for cost-effective reasons. The current COVID-19 pandemic has limited our access to more data.

CONCLUSION:

The prevalence of HPV infection among women with abnormal cervical biopsies was high, despite a small sample size used in this study. Multiparity and early age at sexual intercourse were strong risk factors for HPV infection. Further, the endresult of cervical cancer emphasizes the importance of further national screening programs including those using immunostaining due to its cost-effectiveness. Moreover, including the HPV vaccine in the national immunization program would markedly decrease the burden of this disease.

List of symbols and Abbreviations

HPV: human papillomavirus,
KAUH: King Abdulaziz University Hospital,
HSIL: high-grade squamous intraepithelial lesion,
Pap: Papanicolaou,
WHO: World Health Organization,
IARC: International Agency for Research on Cancer,
SPSS: Statistical Package for Social Sciences,
IRB: Institute of Research Board,
CITI: Collaborative Institutional Training Initiative,
NIH: National Institute of Health,
CIN: cervical intraepithelial neoplasia

ACKNOWLEDGEMENTS:

The authors thank the data collectors Dr. Zainab Slais, Dr. Ghazi Alfuraydi, Dr. Feras Alamir, and Dr. Eman Alrumaih.

REFERENCES:

- [1] Alhamlan FS, Al-Qahtani AA, Al-Ahdal MN. Current studies on human papillomavirus in Saudi Arabia. *J Infect Dev Ctries*, 2015; 9:571-576.
- [2] Palefsky J. 2018. UpToDate [ONLINE] Available at: https://www.uptodate.com/-contents/-human-papillomavirus-infections-epidemiology-and-disease-associations?source=history_widget. [Accessed 08 November 2018].
- [3] Al-Ahdal MN, Al-Arnous WK, Bohol MF, Abuzaid SM, Shoukri M, Elrady KS, Firdous N, Aliyan R, Taseer R, Al-Hazzani AA, Al-Qahtani AA. Human papillomaviruses in cervical specimens of women residing in Riyadh, Saudi Arabia: a hospital-based study. *J Infect Dev Ctries*, 2014; 8:320-325.
- [4] Turki R, Sait K, Anfinan N, Sohrab SS, Abuzenadah AM. Prevalence of human papillomavirus in women from Saudi Arabia. *Asian Pac J Cancer Prev*, 2013;14:3177-3181.
- [5] Gothwal M, Nalwa A, Singh P, Yadav G, Bhati M, Samriya N. Role of cervical cancer biomarkers p16 and Ki67 in abnormal cervical cytological smear. *J Obstet Gynaecol India*, 2021; 71:72-77.
- [6] Chen CC, Huang LW, Bai CH, Lee CC. Predictive value of p16/Ki-67 immunocytochemistry for triage of women with abnormal Papanicolaou test in cervical cancer screening: a systematic review and meta-analysis. *Ann Saudi Med*, 2016; 36:245-251.
- [7] Yu L, Fei L, Liu X, Pi X, Wang L, Chen S. Application of p16/Ki-67 dual-staining cytology in cervical cancers. *J Cancer*, 2019; 10:2654-2660.
- [8] Ordi J, Sagasta A, Munmany M, Rodríguez-Carunchio L, Torné A, del Pino M. Usefulness of p16/Ki67 immunostaining in the triage of women referred to colposcopy. *Cancer Cytopathol*, 2014; 122:227-235.
- [9] McQuillan G, Kruszon-Moran D, Markowitz LE, Unger ER, Paulose-Ram R. 2017. Products - Data Briefs - Number 280. Prevalence of HPV in Adults Aged 18–69: United States, 2011–2014. [ONLINE] Available at: <https://www.cdc.gov/nchs/products/databriefs/db280.htm> [Accessed 27 June 2021].
- [10] Louie KS, De Sanjose S, Diaz M, Castellsague X, Herrero R, Meijer CJ, Shah K, Franceschi S, Muñoz N, Bosch FX. Early age at first sexual intercourse and early pregnancy are risk factors for cervical cancer in developing countries. *Br J Cancer*, 2009; 100:1191-1197.
- [11] Green J, De Gonzalez AB, Smith JA, Franceschi S, Appleby P, Plummer M, Beral V. Human papillomavirus infection and use of oral contraceptives. *Br J Cancer*, 2003; 88:1713-1720.