Relation between Human Papilloma Virus Infection and Spontaneous Miscarriage

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ABSTRACT:

BACKGROUND:

Approximately one in four women will have an early pregnancy failure during her lifetime. In pregnant women or in their partners, human papillomavirus infection can be considered as a risk of preterm birth, miscarriages and virus transmission to the newborn.

OBJECTIVE:

To disclose the prevalence of high risk human papillomavirus infection in women suffering from spontaneous miscarriage .

PATIENTS AND METHODS:

A case - control study included 100 pregnant women ,age (20-42 years old) ,50 full term pregnant (Gestation age 37-40 week) delivered viable full term pregnancy, and 50 pregnant women experienced spontaneous miscarriage (Gestation age 8-24week), cervical swab was done in the labor ward of Al-Yarmouk Teaching Hospital from all participants, and the samples were taken to a private lab for high risk human parvovirus detection. The study period was from 1st of March to 30th of November 2017.

RESULTS:

The overall prevalence of high risk human papillomavirus is 7%, the highest proportion of study patients with high risk human papillomavirus infection ended with miscarriage (71.4%), while in those with viable full term pregnancy were (28.6%). A number of risk factors which increased prevalence of high risk human papillomavirus hrHPV infection were detected as previous history of miscarriage, smoking, and history of oral contraceptives use.

CONCLUSIONS:

High risk human papillomavirus prevalence was higher in pregnancy with spontaneous miscarriage compared to women experience a normal full term pregnancy.

KEYWORDS: Miscarriage, high risk human papillomavirus (hrHPV), Pregnancy.

INTRODUCTION:

Human papillomaviruses (HPV) are common viruses, they are small double stranded DNA viruses that infect mucosae and cutaneous surfaces and cause a variety of lesions ranging from common warts to cervical cancer. Risk factors include sexual behavior of the partner, multiple partners and immune status[(1).]

Most HPV infections are transient and asymptomatic and cause no clinical problems, 70% of women with new cervical HPV infection will clear the infection within one year, and approximately 90% within two years.

Some HPV infections, however, can persist for many years. Persistent infections with high-risk HPV types can lead to cell changes that, if untreated, may progress to cancer [(2)]. Sexually transmitted HPV types fall into two categories, first one; **Low-risk HPVs**, which do not cause cancer but can cause skin warts (technically known as condylomata acuminata) on or around the genitals and anus, for example HPV types 6 and 11, cause 90% of all genital warts**.** Second one; **High-risk HPVs**(hrHPV) which can cause cancer. Two of them, HPV types 16 and 18, are responsible for most HPV-caused cancers [(3)]. HPV vaccination can reduce the risk of infection by the HPV types targeted by the vaccine.

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The Food and Drug Administration (FDA) has approved three vaccines to prevent HPV infection: Gardasil®, Gardasil® 9, and Cervarix®. These vaccines provide strong protection against new HPV infections, but they are not effective at treating established HPV infections or disease caused by HPV [4].

HPV vaccine is not recommended for use during pregnancy [5].

Spontaneous miscarriage is defined as the spontaneous loss of a pregnancy prior to viability, taken legally in the UK as a gestation date of 23 weeks and six days. Beyond this, fetal demise is classified as stillbirth . Ten percent to 15% of clinically recognized pregnancies end in spontaneous miscarriage and the total pregnancy loss is estimated to be 30% to 50% of all conceptions [6].

The most frequent cause of spontaneous miscarriage is fetal chromosome abnormalities. Other implicated causes of miscarriage include Maternal disease: antiphospholipid syndrome, diabetes, thyroid disease, drugs: methotrexate, some antiepileptic drugs ,Uterine abnormalities and Infection[7].

PATIENTS AND METHODS:

Study Design, Setting and Data Collection Time

This is a case - control study that was conducted at the labor ward of Al-Yarmook Teaching Hospital from the 1st of February till the 30th of December 2017.

Study Population and Sample Size

This study included 50 pregnant women with viable full term pregnancy, and 50 pregnant women with spontaneous miscarriage. All participants were subjected to hrHPV screen from their cervix sections.

Exclusion criteria

All of the following were excluded from the study:

1. Age < 20 years old.
2. Patients with PROM.
3. Pregnant women with preterm labor (G.A < 37 completely weeks) or post term labor (G.A > 42 weeks).
4. Multiple pregnancy
5. Any medical disease during pregnancy (e.g. HPT, DM … etc.).
6. Any congenital abnormality of the baby (either diagnosed by U/S or after labor).
7. History of recurrent miscarriage (three or more).

Data Collection Tools

A questionnaire form had been applied to collect the needed information from the participants through direct interview with the patients. It included socio – demographic data (age and place of residence), past obstetrical history (previous full term delivery and previous miscarriage), certain risk factors of HPV infection (smoking, duration of marriage, number of marriages, and history of OCP ingestion) and other information about current miscarriage.

HPV test technique

HPV test was done by performing PCR test. The procedure was explained for each woman. In private room ,patient lie on the examination table . A Cusco's speculum, which was not lubricated is gently inserted to open the vagina ,then taking a sample using cyto-brush from the cervical transformation zone rotated three times in counter clockwise direction, and then the brush removed from the canal and immersed into a fixative jar containing one ml of DNAzole (Invitrogen),each sample labeled with the patient name ,and then placed in the refrigerator at -2 degree centigrade. The PCR assay is based on qualitative detection of 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).

Statistical analysis

Statistical analysis was performed using SPSS windows version 23 Software. Suitable tables and graphs were used to describe the data. Chi square test was used to test qualitative and frequency data and to find any relations between many risk factors and the outcome. P value < 0.05 was considered significant.

RESULTS:

Age and Residence

The distribution of study patients in relation to age and residence is shown in table (1).

Study patient's age was ranging from 20 to 42 years with a mean of 28.4 years and standard deviation (SD) of ± 5.69 years. The highest proportion of study patients was found in age group 20 – 29 (60%).

Regarding residence, the highest proportion of study patients was from urban area (63%).

Table 1: Distribution of study patients in relation to age and residence

Variable	No. (n=100)	Percentage (%)
Age (Years)		
20 – 29	60	60.0
30 – 39	35	35.0
≥ 40	5	5.0
Residence		
Urban	63	63.0
Rural	37	37.0

Previous obstetrical history

Table .2 shows the distribution of study patients by previous obstetrical history.

About two thirds of the study patients (65%) had a previous history of full term pregnancy and most of them (84%) had no history of previous miscarriage.

Table 2: Distribution of study patients by previous obstetrical history

Previous obstetrical history	No. (n=100)	Percentage (%)
Previous full term pregnancy		
YES	65	65.0
NO	35	35.0
Previous miscarriage		
YES	16	16.0
NO	84	84.0

Risk factors for hrHPV infection:

The distribution of study patients by risk factors for hrHPV infection is shown in table (3). Most of the study patients (93%) were not smokers and 92% of them were not married before. Regarding duration of marriage, the highest proportion of the study patients had married for not more than five years (57%).Concerning OCP ingestion, about two thirds of the study patients (67%) had no history of OCP ingestion.

Table 3: Distribution of study patients by risk factors for hrHPV infection

Variable	No. (n=100)	Percentage (%)
Smoking		
YES	7	7.0
NO	93	93.0
Duration of Marriage (Years)		
≤ 5	57	57.0
> 5	43	43.0
Number of marriages		
First	92	92.0
Second	7	7.0
Third	1	1.0
Current OCP use		
YES	33	33.0
NO	67	67.0

HrHPV infection

Prevalence of hrHPV infection in study groups.

Prevalence of hrHPV infection in cervical tissue of healthy full term pregnancy was done in 50 pregnant women of the total 100 included study group, hrHPV prevalence was 4%, while the prevalence of hrHPV in the cervical tissue of patients with miscarriage was 10%, figure (1). It is obvious that prevalence of hrHPV infection among women ended with miscarriage during pregnancy was higher than that among control group (10% versus 4%).

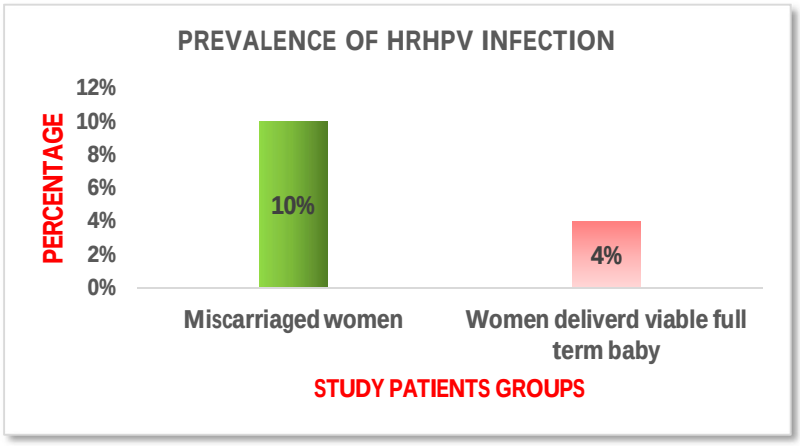

Figure 1: Prevalence of hrHPV infection in full term pregnancy compared to miscarriage.

Influence of the gestational age of samples collection on the prevalence of hrHPV.

The prevalence of hrHPV infection according to the time of miscarriage, we noticed that highest prevalence of hrHPV infection presented in women who had miscarriage that occurred between 12+ – 24 weeks of gestation (12%), while the prevalence of hrHPV in the late first trimester was (8%), Figure 2.

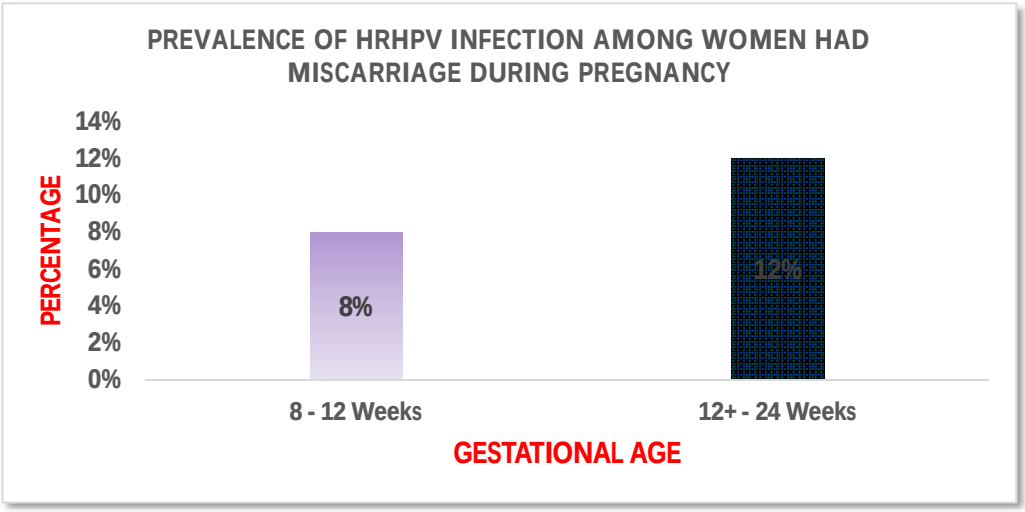

Figure 2: Influence of the gestation age of samples collection on the prevalence of hrHPV

HrHPV prevalence in pregnancy with adverse outcome (miscarriage) and comparison to those with full term pregnancy.

The highest proportion of study patients with hrHPV infection ended with miscarriage (71.4%), but the association between adverse pregnancy outcome with hrHPV infection in relation to those full term with hrHPV infection was statistically not significant (P=0.436), table (.4).

Table .4: Comparison between pregnancy with adverse outcome and hrHPV infection in normal full term pregnancy.

hrHPV infection	Pregnancy Outcome		Total (%) N= 100	P - value
	Full term delivery (%) N= 50	Spontaneous Miscarriage (%) N= 50		
YES	2 (28.6)	5 (71.4)	7 (7.0)	0.436
NO	48 (51.6)	45 (48.4)	93 (93.0)	

Chi-sequare test, significant at 0.05 level..

Comparison between hrHPV infection in relation to age and residence.

The association between hrHPV infection in relation to age and residence is shown in table (5). We noticed that there was no statistically significant association ($P \geq 0.05$) between hrHPV infection and both age and residence.

Table .5: Comparison of hrHPV infection with age and residence

Variable	hrHPV infection		Total (%) n=100	P- value
	YES (%) N=7	NO (%) N= 93		
Age				
20 – 29	5 (8.3)	55 (91.7)	60 (60.0)	0.73
30 - 39	2 (5.7)	33 (94.3)	35 (35.0)	
≥ 40	0 (0)	5 (100.0)	5 (5.0)	
Residence				
Urban	3 (4.8)	60 (95.2)	63 (63.0)	0.418
Rural	4 (10.8)	33 (89.2)	37 (37.0)	

Chi-square test ,Significant at 0.05 level,

Comparison between hrHPV infection and previous obstetrical history

Table .6 shows the association between hrHPV infection and previous obstetrical history. It is obvious that the proportion of study patients with previous history of miscarriage had more possibility to have hrHPV infection (25%) than those without previous history of miscarriage (3.6%), and the association between hrHPV infection and previous history of miscarriage was statistically significant (P=0.012).

Regarding previous history of full term pregnancy, there was no statistical significant association (P=0.97) between hrHPV infection and previous history of full term pregnancy.

Table 6:Comparison between hrHPV infection and previous obstetrical history

Previous obstetrical history	hrHPV infection		Total (%) n=100	P- value
	YES (%) N=7	NO (%) N= 93		
Previous full term pregnancy				
YES	5 (7.7)	60 (92.3)	65 (65.0)	**0.97**
NO	2 (5.7)	33 (94.3)	35 (35.0)	
Previous miscarriage				
YES	4 (25.0)	12 (75.0)	16 (16.0)	**0.012**
NO	3 (3.6)	81 (96.4)	84 (84.0)	

Chi-square test ,Significant at 0.05 level,

Association between hrHPV infection and certain risk factors

The association between hrHPV infection and certain risk factors is shown in table (7).

This study showed that the highest proportion of hrHPV infection prevalence was seen among smokers' study patients (42.9%) with a significant association (P=0.007) between smoking and prevalence of hrHPV infection.

The highest proportion of hrHPV infection was seen among study patients who had history of OCP (15.2%) with a significant association (P = 0.038) between history of OCP use and hrHPV infection prevalence.

No significant association (P ≥ 0.05) was seen between both of duration and number of marriages with prevalence of hrHPV infection.

Table .7: Association between hrHPV infection and certain risk factors

Risk factors	hrHPV infection		Total (%) n=100	P - value
	YES (%) N=7	NO (%) N= 93		
Smoking				
YES	3 (42.9)	4 (57.1)	7 (7.0)	**0.007**
NO	4 (4.3)	89 (95.7)	93 (93.0)	
Duration of marriage (Years)				
≤ 5	4 (7.0)	53 (93.0)	57 (57.0)	**1.00**
> 5	3 (7.0)	40 (93.0)	43 (43.0)	
Number of marriages				
First	5 (5.4)	87 (94.6)	92 (92.0)	**0.066**
Second	2 (28.6)	5 (71.4)	7 (7.0)	
Third	0 (0)	1 (100.0)	1 (1.0)	
History of OCP use				
YES	5 (15.2)	28 (84.8)	33 (33.0)	**0.038**
NO	2 (3.0)	65 (97.0)	67 (67.0)	

Chi-square test ,Significant at 0.05 level,

DISCUSSION

HPV is one of the most common sexually transmitted viruses in the world, and it is evident that many different types of HPV existed due to the advent of recombinant DNA technology, some of which were carcinogenic. Recent evidence suggested that HPV infection may affect fertility and alter the efficacy of assisted reproductive technologies [7,8]. In pregnant women or in their partners, HPV infection can be considered as a risk of preterm birth, miscarriages and virus transmission to the newborn . HPV detection rates range widely from 6 to 65% and the results are controversial [9,10].

In our study the prevalence of hrHPV infection was (4%) in normal full term pregnancies, in comparison to this, miscarriages found to have higher hrHPV prevalence rates (10%), but this increment in hrHPV infection was not significant compared with women delivered viable full term baby. In other studies, HPV infection prevalence was reported in a quantitative analysis when 14 470 pregnant women were analyzed and they found that the HPV infection prevalence in women delivered viable full term baby was 35.5% and 29.6% in population from Latin America and USA respectively. The Asian population that represented by China, Japan, and Korea showed that the cervical HPV infection prevalence varied between 10.1% and 36.2%. European population showed that the HPV infection prevalence varied between 2.2% and 36.6%, with a summary estimate of 11% [11]

Also, in our study we found that the highest proportion of study patients with hrHPV infection were ended with miscarriage (71.4%), but the association between pregnancy outcome and hrHPV infection was statistically not significant. We can compare this result with studies that investigated the linkage between HPV infection and miscarriages. The same result was found by a case-control study performed in Mexico in 2013 and reported that HPV prevalence was higher in women experiencing spontaneous abortion than HPV prevalence in the control group but the difference did not reach the statistical significance [12] . Another study performed in Poland, 2011 and reported that the association between HPV and miscarriages was not statistically significant [13].

In contrary, other studies found a significant higher prevalence of HPV infection among women who miscarried than among women who delivered a viable full term baby as an in vitro study in USA 2008 and proved that trophoblasts transfected with plasmids harboring HPV16 genome undergo apoptosis at rates three to six times higher than trophoblasts transfected with empty plasmids. This could be responsible for dysfunctions in placenta, reduction of embryo ability to invade the uterine wall and finally lead to miscarriages in the earlier stages of pregnancy[4].

The time of samples collection during pregnancy is thought to have an influence on hrHPV prevalence [15]. Our analysis shows that the second trimester found to have the highest hrHPV prevalence followed by the first trimester (12% versus 8%), this finding was also reported in a cross sectional study performed in Japan 2013 which reported highest prevalence during the second trimester [16]. On the other hand, multiple hrHPV infection were observed by Yamasaki et al.in 2011 and hrHPV were found to be selectively increase in first trimester[17].

In respect to the choice of hrHPV detection methods. we have choose PCR which is the most sensitive ,specific , and widely used method for the detection of cervicovaginal HPV[18]

Furthermore, our analysis showed that hrHPV infection was significantly higher among women with a positive previous history of miscarriages than those without history of previous miscarriages (25% versus 3.6%, P=0.012).

This result is similar to a study conducted in Morocco in 2007-2008 to assess the correlation between HPV infection and potential risk factors and reported that histories of abortion are significantly associated with high HPV prevalence[19] .

In contrary, a result found by a case-control study that was carried out in Italy in 2013 which explored the possible role of HPV in recurrent miscarriages, comparing HPV prevalence in women with history of multiple miscarriages and in women with at least one pregnancy at term and with no history of spontaneous miscarriages and showed that HPV prevalence was higher in the group of women without a history of recurrent miscarriages [20] .

In the present work, we find that HPV infection was significantly higher among currently smokers than non-smokers (42.9% versus 4.3% P=0.007). This result is consistent with the findings obtained by a study of women with prevalent HPV16 and/or HPV18 infections and found that baseline viral load was statistically significantly higher among current smokers than among never smokers [21]. A possible mechanism for the association between cigarette smoking and HPV DNA load is that cell proliferation and turnover in the transformation zone of the cervix may increase by smoking. This may leads to an increase in replication of HPV DNA and/or production of infectious virus as HPV uses the host cell DNA replication machinery for its own replication. Another possible mechanism is that smoking causes weakening of cellular immune response and this may increase viral load [21]. And this fact is supported by studies showing that smoking has adverse effects on both systemic and local immunity [22].

Also this analysis found that HPV infection was significantly higher among oral combined pills (OCP) current users than OCP non-users (15.2% versus 3%, P=0.038). A possible explanation for this association might be due to the fact which proved by a number of hypotheses when reported that the use of OCP is associated with an increased incidence of cervical ectropion, which means that the site where HPV infection preferentially induces neoplastic lesions. Estrogen and progesterone may also affect cervical cells directly, increasing cell proliferation and stimulating transcription of HPVs [23].

A similar result found by a study investigated the sero-epidemiology of HPV-16 infection in the United States in 2002 by using a population-based survey which reported that HPV-16 sero-positivity was significantly associated with OCP use [24].In contrast, a review from studies eligible for inclusion criteria in 2003 showed that there was no clear evidence for an effect of duration of OCP use on HPV positivity [25].

CONCLUSION:

High risk HPV prevalence was higher in pregnancy with adverse outcomes such as spontaneous miscarriage compared to women experience a normal full term pregnancy.

REFERENCES:

1. Hinten F, Meeuwis KA, Van Rossum MM, De Hullu JA. HPV-related (pre) malignancies of the female anogenital tract in renal transplant recipients. Critical reviews in oncology/hematology. 2012 Nov 30; 84(2):161-80.
2. Rositch AF, Koshiol J, Hudgens MG, Razzaghi H, Backes DM, Pimenta JM. Patterns of persistent genital human papillomavirus infection among women worldwide: a literature review and meta-analysis. International journal of cancer. 2013 Sep 15; 133(6):1271-85.
3. Centers for Disease Control and Prevention (CDC. Human papillomavirus-associated cancers -United States, 2004-2008. MMWR. Morbidity and mortality weekly report. 2012 Apr 20; 61:258.
4. Schiller JT, Castellsagué X, Garland SM. A review of clinical trials of human papillomavirus prophylactic vaccines. Vaccine. 2012 Nov 20; 30: F123-38.
5. Meites E. Use of a 2-dose schedule for human papillomavirus vaccination—updated recommendations of the Advisory Committee on Immunization Practices. MMWR. Morbidity and mortality weekly report. 2016 Dec 16;65(49):1405-1408
6. Bressler LH, Correia KF, Srouji SS, Hornstein MD, Missmer SA. Factors associated with second-trimester pregnancy loss in women with normal uterine anatomy undergoing in vitro fertilization. Obstetrics & Gynecology. 2015 Mar 1; 125(3):621-7.
7. Catriona MS. Spontaneous miscarriage.In Dewhurst's Textbook of Obstetrics & Gynecology, 8th Edition,Blackwell publishing Ltd ;London;2012. p 53-55
8. Souho T, Benlemlih M, Bennani B. Human papillomavirus infection and fertility alteration: a systematic review. PLoS One. 2015 May 18; 10(5).
9. Pereira N, Kucharczyk KM, Estes JL, Gerber RS, Lekovich JP, Elias RT. Human papillomavirus infection, infertility, and assisted reproductive outcomes. Journal of pathogens. 2015 Sep 30;2015:8.

10. Worda C, Huber A, Hudelist G et al. Prevalence of cervical and intrauterine human papillomavirus infected in the third trimester in asymptomatic women," Journal of the Society for Gynecologic Investigation.2005; vol. 12, no. 6, pp. 440–444

11. Ambühl L, Baandrup U, Dybkær K, Blaakær J, Uldbjerg N, Sørensen S. Human Papillomavirus Infection as a Possible Cause of Spontaneous Abortion and Spontaneous Preterm Delivery. Infectious Diseases in Obstetrics and Gynecology. 2016; 2016:1-19.

12. Conde-Ferraez L, Chan May A,Carrillo-Martinez J, Ayora-Talavera G,Gonzalez-Losa M. Human papillomavirus infection and spontaneous abortion: acase-control study performed in Mexico.European Journal of Obstetrics &Gynecology and Reproductive Biology.2013;170(2):468:473 .

13. Skocynski M, Gozdzicka-jozefik A, Kwasniewska A. Prevalence of human papillomavirus in spontaneously aborted products of conception. Acta Obstetricia ET Gynecological Scandinavica. 2011; 90(12):1402-1405.

14. Gomez L, Ma Y, Ho C, McGrath C, Nelson D, Parry S. Placental infection with human papillomavirus is associated with spontaneous preterm delivery. Human Reproduction. 2008; 23(3):709-715.

15. Schneider A, Hotz M, Gissmann L. "Increased prevalence of human papillomaviruses in the lower genital tract of pregnant women," International Journal of Cancer, 2007 Aug 15 ;40(2):198-201.

16. Kim YH, Park JS, Norwitz ER et al. "Genotypic prevalence of human papilloma virus infection during normal pregnancy: across sectional study" Journal of Obstetrics and Gynecology.2014 Jan 1;40(1):200-207.

17. Depuydt CE, Boulet GA, Horvath CA ,Benoy IH ,Vereecken AJ ,Bogers JJ ,Comparison of MY 09/11 consensus PCR and type-specific PCRs in the detection of oncogenic HPV types .Journal of cellular and molecular medicine.2007 Jul 1;11(4):881-91 .

18. Yamasaki k, Miura k, Shimada T et al. "Epidemiology of human papillomavirus genotypes in pregnant Japanese women," Journal of Human Genetics. 2011 Apr 1;56(4):313-315 .

19. Bennani B, Bennis S, Nejjari C, Ouafik L, Melhouf M, El Rhazi K . Correlates of HPV: a cross-sectional study in women with normal cytology in north-central Morocco. The Journal of Infection in Developing Countries. 2012 Nov 30; 6(07):543-550.

20. Ticconi C, Pietropolli A, Fabbri G, Capogna M, Perno C, Piccione E. Recurrent Miscarriage and Cervical Human Papillomavirus Infection. American Journal of Reproductive Immunology. 2013 Nov 1;70(5):343-346.

21. Xi L, Koutsky L, Castle P, Edelstein Z, Meyers C, Ho J . Relationship Between Cigarette Smoking and Human Papilloma Virus Types 16 and 18 DNA Load. Cancer Epidemiology Biomarkers & Prevention. 2009 Dec 1; 18(12):3490-3496.

22 Sopori M. Science and society: Effects of cigarette smoke on the immune system. Nature Reviews Immunology. 2002 May 1; 2(5):372-377.

23 De Villiers E. Relationship between steroid hormone contraceptives and HPV, cervical intraepithelial neoplasia and cervical carcinoma. International Journal of Cancer. 2003 Mar 1; 103(6):705-708

24 Stone K, Karem K, Sternberg M, McQuillan G, Poon A, Unger E . Seroprevalence of Human Papillomavirus Type 16 Infection in the United States. The Journal of Infectious Diseases. 2002 Nov 15; 186(10):1396-1402.

25 Green J, de Gonzalez BA, Smith J, Franceschi S, Appleby P, Plummer M . Human papillomavirus infection and use of oral contraceptives. British Journal of Cancer. 2003 Jan 02; 88(11):1713-1720.