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Article

# Detection of HPV-16 in Cervical Swab in Woman with Recurrent Pregnancy Loss by Real-Time PCR

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**ABSTRACT**
Human Papilloma Virus is one of the most common sexually transmitted infections. It causes a series of neoplasms, including cervical, vulvar, vaginal, penile, anal, and head-and-neck cancers. The current study aimed to determine the role of human papillomavirus and its relationship to the incidence of recurrent miscarriages. A cross-sectional hospital-based study was carried out in Kirkuk City on 114 women who had recurrent miscarriages and included 36 women with previous two or more normal pregnancies as controls. Deep vaginal swabs were collected from all women enrolled in the study according to the standard procedure of vaginal swab sampling until PCR extraction and amplification tests were done by real-time PCR for qualitative and quantitative. The study showed that 10.53% (12 of 114) of RPL patients tested positive by PCR, while none of the control patients had PCR +ve results. The mean HPV DNA load detected by RT-PCR from the RPL group (n=12) was 10.95 copies/cell (range 7.665–15.75 copies/cell). Furthermore, it demonstrated that the high mean of HPV DNA load (13.724 copies/cell) was recorded among RPL with a number of abortions (5 and more). It was concluded that human papillomavirus is highly related to recurrent abortion.

**Keywords:** RPL; HPV DNA; E6 protein; RT-PCR

## INTRODUCTION

The term "recurrent miscarriage" refers to the loss of two or more pregnancies in a row before the 24th week of gestation, regardless of whether the woman has previously given birth to a healthy child[1]. In half of all cases of early pregnancy loss, chromosomal abnormalities in the developing fetus are suspected. It is not uncommon for women to become pregnant quickly after giving birth, especially if they have already had a child or are infected with an infection such as cervicitis, vaginitis, or HIV. It is also possible to get pregnant quickly after giving birth. Another significant risk factor is exposure to environmental contaminants like arsenic, lead, and organic solvents[2]. *Herpes simplex viruses 1 and 2, cytomegalovirus, dengue virus, zika virus, adenovirus*, and the adeno-associated virus are all human viruses that can infect the placenta, trophoblast or cytotrophoblasts following viremia or an ascendant infection. Women and their fetuses can contract these diseases while pregnant[3-4-5]. Previous research has suggested that the Human papillomavirus, also known as HPV, may influence the outcome of a pregnancy[3-6-7]. Additionally, HPV is one of the most common sexually transmitted infections, causing a variety of cancers, including cervical, vulvar, vaginal, penile, and anal cancers (STIs). Aside from that, it is a common cause of genital warts and extremely severe and

etiologically linked HPV recurrent respiratory papillomatosis[8-9-10-11]. Human papillomavirus types 6, 11, 40, 42, 43, 44, and 54 are responsible for genital warts, low-grade intraepithelial squamous lesions, and laryngeal papillomatosis. These kinds of cancers are considered to be of low risk[11-12]. There are seven HPV nonstructural proteins encoded in the virus's genome, and they are known collectively as nonstructural proteins E1, E2, E3, E4, and E5. Viral replication and transcription cannot take place without the presence of these proteins[8]. The E6 and E7 proteins are considered to have a high risk of cancer because of their role in preventing cell differentiation. Numerous cellular processes have been linked to the disruption of normal epithelial differentiation, the inability to apoptosis, DNA synthesis, and cell cycle control[8-13-14]. The current study aimed to determine the role of human papillomavirus and its relationship to the incidence of recurrent miscarriages. The current study showed that HPV DNA can infect the squamous epithelium of the cervix and that HPV infection can occur in the first trimester of pregnancy.

## MATERIALS AND METHODS

A cross-sectional hospital-based study was carried out in Kirkuk City from the beginning of September 2021 to the end of February 2022 on 114 women who attended a Gynecological and Pediatric Hospital aged 20-40. The study also included 20 women with two or more normal pregnancies, and no history of abortion served as controls. Patients with chronic diseases, clinical evidence of genitourinary infection, women suffering from any bacterial or viral infection related to abortion, such as *Rubella, cytomegalovirus*, *Human Herpes Virus*, and *Toxoplasma parasites*, were excluded from the study. Deep vaginal swabs were collected from all women enrolled in the study according to the standard procedure of vaginal swab sampling; swabs were inserted in tubes containing viral transport medium and stored in deep freeze until PCR extraction and amplification tests were done by real-time PCR for qualitative and quantitative, HPV DNA detection was determined by real-time PCR using broad-spectrum HPV-specific GP5+/6+ primers.

## RESULTS

The study showed that 10.53% (12 of 114) of RPL patients were positive by PCR, while none of the control patients had PCR +ve results, Table 1.

| HPV 16 PCR | Studied groups | | | |
|---|---|---|---|---|
| | Positive | | Negative | |
| | No. | % | No. | % |
| **Positive** | 12 | 10.53 | 0 | 0 |
| **Negative** | 102 | 89.47 | 20 | 100 |
| **Total** | 114 | 100 | 20 | 100 |

**Table 1: Comparison between ELISA and PCR in detecting HPV 16 in RPL women.**

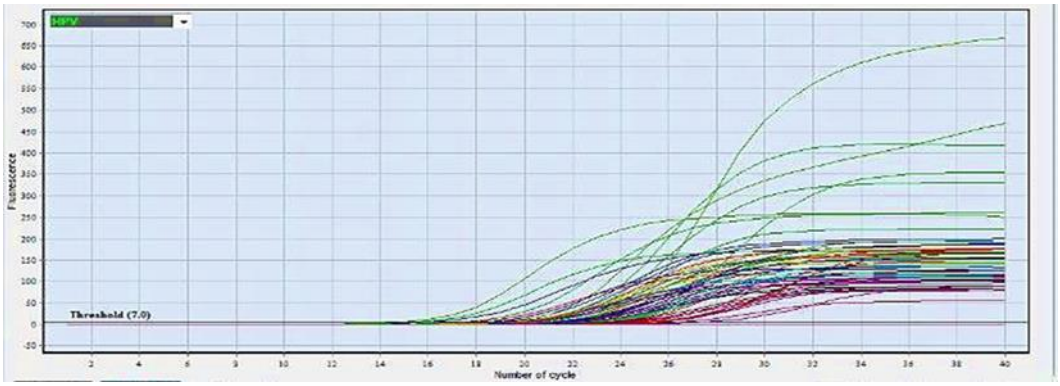


**Figure 1: Dependence of FAM/HEX /CY5.5 channel fluorescence on cycle number.**

For example, 39.02 percent of women with a history of three miscarriages were in the 30-39 year age range, 67.65 percent of women with a history of four miscarriages were under 30 years old, and 46.15 percent of women with a history of five miscarriages or more were over 40 years old (see Table 2). The outcome was extremely important. (p: 0.0001).

| **Age groups (years)** | **N. of miscarriage** | | | | | |
|---|---|---|---|---|---|---|
| | Three | | Four | | Five and more | |
| | No. | % | No. | % | No. | % |
| **Less than years** | 16 | 39.02 | 23 | 67.65 | 11 | 28.21 |
| **30-39** | 19 | 46.34 | 8 | 23.53 | 10 | 25.64 |
| **≥ 40** | 6 | 14.63 | 3 | 8.82 | 18 | 46.15 |
| **Total** | 41 | 100 | 34 | 100 | 39 | 100 |

**Table 2: Relation of age with number of recurrent pregnancy loss.**

A real-time PCR assay using HPV-specific GP5+/6+ primers determined the number of HPV DNA copies in the 12 RPL women who tested positive. In the RPL group, the mean HPV DNA load detected by RT-PCR in deep vaginal swabs was 10.95 copies/cell (range 7.665–15.75 copies/cell), according to quantitative results.

| | |
|---|---|
| ***No. of +ve PCR cases*** | *12* |
| ***Mean*** | *10.95* |
| ***SD*** | *2.84* |
| ***Minimum*** | *7.655* |
| ***Median*** | *10.821* |
| ***Maximum*** | *15.75* |

**Table 3: The HPV DNA Load among RPL women.**

The study demonstrated that the high mean of HPV DNA load (13.724 copy/cell) was recorded among RPL with a number of abortions (5 and more), and the lowest mean (7.953 copy/cell) was RPL women with 3 times aborted previously as in Table 4.

| No of miscarriage | No. | HPV DNA load (copy/cell) | | P. value |
|---|---|---|---|---|
| | | Mean | SD | |
| **3** | 4 | 7.953 | 0.33 | 0.0001 |
| **4** | 3 | 10.347 | 0.86 | |
| **5 and more** | 5 | 13.724 | 1.68 | |

**Table 4: Relation of HPV DNA Load with number of miscarriage**

Table 5 the study demonstrated that the high mean of HPV DNA load (11.186 copy/cell) was recorded among RPL with a history of stillbirth, and the lowest mean (8.51 copy/cell) was RPL women who have no history, as in Table 5

| Still-births | No. | HPV DNA load (copy/cell) | | P. value |
|---|---|---|---|---|
| | | Mean | SD | |
| **No** | 10 | 8.51 | 2.19 | 0.001 |
| **Yes** | 3 | 11.186 | 2.32 | |

**Table 5: Relation of HPV DNA Load with history of stillbirth**

## DISCUSSION

The study showed that 10.53% (12 of 114) of RPL patients were positive by PCR, while none of the control patients had positive PCR +ve results. According to Bruno et al. [15], papillomavirus DNA can infect the placenta, and that placental HPV infection can occur as early as the first trimester of pregnancy. These findings are in agreement with the previous findings. These findings are supported by Hammad et al. [16], who demonstrated that women who have experienced recurrent miscarriages have a higher prevalence of human papillomavirus (HPV). Tenti et al. [17] reported that the modulated hormonal environment and immune response enhance the presence and persistence of HPV infection. These findings are consistent with the findings reported by these researchers. According to Christiansen et al. [18], recurrent miscarriage is defined as having two or more prior miscarriages, whereas earlier definitions included having three or more prior miscarriages[19].The findings of our most recent research align with Hu et al. [20], who demonstrated that the incidence rate of spontaneous abortion increases with the increase in the age of pregnant women. Women who become pregnant later in life experience a decline in ovarian function and an increase in the incidence of abnormal chromosomal abnormalities, both of which contribute to an elevated risk of aneuploidy in the developing embryo. As a result, the volume of the decidua is reduced, and the endometrium's receptivity is decreased, which leads to a higher rate of pregnancy abortion. According to the findings of a study that was carried out in China, there is a correlation between the mother's age when she conceived a child and the presence of chromosomal abnormalities. This makes sense, given that the egg deteriorates with age. Galamb et al.[22] found that an abnormal anatomical structure of the uterus

was associated with an increased risk of spontaneous abortion. The uterine cavity's normal structure and local environment can be altered when uterine septum, endometrial polyp, and uterine fibro are present. Consequently, the area available for embryo implantation and endometrial blood flow are reduced. This results in increased resistance to blood flow, which in turn affects embryo implantation and the fetus's growth and development. According to Ticconi et al.[23], there are several distinct kinds of abortions and a variety of factors that can make a pregnancy more difficult in the first three months. One of the most important risk factors is a history of miscarriage in the past, followed by obesity as the second factor, diabetes as the third factor, and the thyroid gland as the fourth factor. Additionally, estrogen plays a significant role in a pregnancy's continuation and maintenance. Also, the implantation process of the fetus in the uterine lining depends on the balance between estrogen and progesterone. This explains the high estrogen in the age group (33-40) years, as most miscarriages and difficulty getting pregnant are due to an imbalance between estrogen and progesterone in the later stages of a woman's life. Progesterone is a hormone produced by the ovaries and plays a role in a woman's ability.It is unclear what the underlying mechanism links viral load and cervical mucous. According to Conde-Ferráez et al. 25, whether or not an increase in viral copy numbers is concurrent with an increase in viral protein expression and production of the virion is largely unknown. Tognon et al. [26] reported findings that were in agreement with ours. For the HPV45-positive sample, qPCR was used to determine the HPV DNA load, and the results indicated that the mean HPV DNA load detected was 7.12 copies per cell. Additionally, various HPV DNA assays were used for qualitative and quantitative analyses. These studies used newly developed PCR-based primers specific for HPV GP5+/6+ that cover a wide spectrum of the virus[27]. Previous studies have found low HPV DNA loads in the outer layer cells of the placenta, known as Hofbauer cells[28] and chorionic villi[29], but the current findings support those earlier findings. Variations in HPV detection methods, differences in risk factors such as a history of cervical dysplasia, genital warts, maternal and gestational ages, other sexually transmitted infections, and variations in other risk factors may all contribute to this variation in HPV prevalence[30]. As You et al. [31]. have discovered, the trophoblasts 3 are subjected to multiple physiological changes when E6 and E7 are introduced into the environment. On E6 and E7, 3A trophoblast cells, they tested the effects of AAV/NEO infection and compared them to the results of AAV/NEO infection, which served as a control. There is a lack of recognition of highly defective endometrial cells, which results in low adhesion of trophoblast cells to the endometrium and the death of trophoblast cells induced by E7. That HPV is linked to miscarriage is backed up by evidence that the virus damages the trophoblast. This hypothesis is supported by the knowledge that a healthy placentation is essential for a physiological pregnancy. In conclusion, the oncogenic component genes of HPV E6 and E7 significantly affect the general cellular characteristics of trophoblasts. These effects include cell survival, the binding of trophoblasts to endometrial cells, proliferation, differentiation, and immortalization. There is a possibility that any of these alterations in trophoblasts could be to blame for a change in the physiology of the trophoblasts and the placenta, which in turn could be a factor in spontaneous abortions. Bober et al.[6] provided evidence that the virus is associated with adverse effects on pregnancy. The virus uses the HPV elevated progesterone serum concentration to regulate its life cycle and activity. This is why the HPV LCR's primary function is to exert an effect on the processes of transcription and replication by generating signals that control the activity of other viral genes. In addition, the immune system cannot respond effectively while a woman is pregnant, which further contributes to the spread of infections. According to Condrat et al. [32], HPV infection during pregnancy negatively affects both the mother and the infant, increasing the risk of severe

pregnancy complications such as spontaneous abortion, premature birth, preeclampsia, and intrauterine growth restriction.

## CONCLUSIONS

The current study showed that HPV DNA can infect the squamous epithelium of the cervix and that HPV infection can occur in the first trimester of pregnancy. There is a close relationship between the number of abortions and the mother's age. Young women have higher HPV DNA levels than older women below 45 years.

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**Conflicts of Interest:** None

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