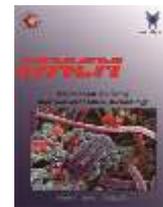




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Frequency of Human papilloma virus among pregnant women by PCR technique

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ABSTRACT

Today, it has been confirmed that the viral infections play an important role in premature delivery and abortion. This study was carried out to determine the frequency rate of papilloma virus types 16 and 18 in pregnant women and their relationship with premature delivery and abortion. In this study, vaginal secretions were collected from pregnant women who had been referred to the women clinics in Tonekabon, a city in North of Iran. The samples were used to amplify E6 gene of papilloma virus through Polymerase Chain Reaction method. The results were analyzed by χ^2 statistical test. Of 47 tested samples, 14 cases were infected with type 16 papilloma virus (HPV16) (29.78%), while 3 cases were infected with type 18 papilloma virus (HPV18) (6.38%). Papilloma virus infection had no significant relationship with abortion and premature delivery. Considering the obtained findings, it appears that a high percentage of the study population was infected with HPV16 virus. Therefore, risk of affliction with abortion and premature delivery can be decreased up to a limit in this region by screening before pregnancy.

1. Introduction

Pregnancy is a complicated process that many factors interfere in the passage of its natural trend. Premature evolution, hormonal activity of embryo, excess expansion of uterus, endometrial hemorrhage and uterine internal infections are of the factors that can induce premature delivery and abortion (Simhan and Caritis, 2007; Mohseni et al., 2013).

There are evidences that viral infections play an important role in abortion. In addition, some evidences suggest that uterus infections play a role

in creation of major complications in nurslings and newborns including myocarditis, encephalitis, pulmonary abnormality and cerebral palsy (Romero and Gomez 2001).

Based on several reports, papilloma viruses available in the vagina cause premature rupture of amniotic membrane. It is thought that they can be considered as a risk factor of abortion. Due to the deplorable difficulties that this sort of deliveries creates for neonates, they are now considered as a global problem (Kotani and Shimada 1998; Hauth et al., 2000). HPVs are DNA, non-enveloped and

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icosahedral symmetry viruses that are able to infect human beings.

They infect human beings through reproduction on the skin surface and mucous membranes (Schiffman and Castle 2003). Approximately, 200 types of these viruses have been identified, of which many cause warts in human beings (Walboomers *et al.*, 1999). Some other types such as HPV16 and HPV18 are considered as the risk factors of cervical, vaginal and anal cancers. (Franco *et al.*, 2001; Burd, 2003; Goldstein *et al.*, 2009; Kahn, 2009; Rampias *et al.*, 2010). More than 30 to 40 types of these viruses are transferred sexually and cause genital warts (Chaturvedi and Maura 2010). Since the prevalence of abortion in Iran is reported to be high and, on one hand, there is no accurate information regarding the carriers of this virus this study was conducted to investigate the relationship between HPV infection and premature delivery and abortion.

2. Materials and Methods

A total of 47 pregnant women referred to the women's clinics located in tonekabon, a city in northern Iran, for a duration of time effective from October, 2012 until February, 2013 were recruited in this study.

The protocol of the study was confirmed by the research committee of the Islamic Azad University-Tonekabon Branch and informed consent was obtained from all participants. Sampling was done by specialized gynecologists and sterile swabs were used to collect vaginal secretions of each individual. Samples were added to one milliliter of transport medium (PBS) and kept in -20°C until DNA extraction. DNA was extracted using Genomic DNA extraction kit (Kiagene-Germany). The amplification capability of extracted DNA was assessed by amplifying a fragment of human Beta-globulin gene. In order to amplify E6 gene of the type 16 and type 18 papilloma viruses, the specific primers were synthesized by Tag Copenhagen Company (Denmark) after extraction from the scientific articles (Zaravinos *et al.*, 2009). (table 1). After provision of mixture of reaction, the samples were placed in the thermal cycler apparatus (Biorod- Germany) with its own specific time and thermal plan (Table 2).

The data were analyzed by SPSS software and χ^2 statistical test, and rate of lower than 0.05 was considered to be significant.

Table 1. sequence of the employed primers to amplify beta-globin gene and types 16 and 18 of papilloma virus.

Primer name	Nucleotide Sequence 5' to 3'	Product size
Beta globin	TCCAACATCAACATCTTGGT	122 bp
Beta globin	TCCCCAATTCTAAGCAGA	
HPV 16	CTGCAAGCAACAGTTACTGCCACG	315 bp
HPV 16	CATACATCGACCGGTCCACC	
HPV 18	AAACTAACTAACACTGGGTTATACA	143 bp
HPV 18	ATGGCACTGGCCTCTATAGT	

Table 2. Thermal and timing plan for amplification of Beta-globin gene and types 16 and 18 of papilloma virus.

	Beta Globin	HVP 16	HVP 18
Pre denaturation	95°C – 5 min	95°C–5 min	95°C – 5 min
Denaturation	95°C – 45 s	94°C – 45 s	95°C – 45 s
Annealing	55°C – 45 s	60°C – 40 s	54°C – 45 s
Extension	72°C – 40 s	72°C – 45 s	72°C – 45 s
Final Extension	72°C – 5 min	72°C–5 min	72°C – 5 min

3. Result

The average age of the participants was 5.9 ± 24.8 years, with maximum and minimum ages of 45 and 18 years old, respectively (Table 3).

Amplification of Beta-globulin, HPV16, and HPV18 led to production of 122 bp, 315 bp and 143 bp fragments, respectively.



Figure 1. Gel electrophoresis PCR products, from left to right No. M: 100 bp size marker, No. 1: Positive control for Beta globulin Fragment (122 bp), No. 2: Sample positive for HPV16, No. 3: Sample positive for HPV18. No.4: Negative control.

Of 47 cases examined, 14 cases (29.78%) were infected with HPV16, while 3 (6.38%) of them were infected with HPV18. The age distribution of infected patients are given in Table 3.

Table 3. Age distribution of patients with HPV16 and HPV18 infections.

age	number	HPV 16	HPV 18
15-20	2	0	0
21-25	11	4	1
26-30	19	5	2
31-35	11	3	0
36-40	3	2	0
41-45	1	0	0
total	47	14	3

Among 47 cases studied, 3 cases had positive history for abortion, while 1 case was positive for premature delivery. All 4 cases were also positive for HPV16 infection. The statistical analysis shows no significant relationship between HPV infection with either abortion and premature delivery.

4. Discussion

In this study, the rate of high-risk papilloma viruses' infection among pregnant women was evaluated to be 36%, which represents a relatively high prevalence. The results show that cervix probably provides an appropriate medium for papilloma virus to infect. Some studies performed by various researchers all around the world have shown that HPV, in addition to the strong reproduction in keratinocyte skin cells, is able to reproduce in trophoblast endometrial cell lines too.

This hypothesis supports the assumption that HPV infected genital organs can pass the infection to the placenta and play a role in premature delivery and abortion.

During the process of pregnancy, physiological changes such as hormonal and immunological fluctuations can positively affect the troublesome caused by HPV infection. Some studies have shown a higher frequency of HPV infection among pregnant women than those who were not pregnant. Liu *et al.* in 2014 conducted a study on the frequency of Papilloma virus among 13640 pregnant and non pregnant women. Based on the results reported by them, the frequencies of infections among pregnant and non-pregnant women were 16% to 82% and 12% to 25%, respectively.

It seems that infection by high risk HPVs, even when with no explicit clinical symptom, can cause difficulties during pregnancy and lead to premature delivery and abortion by transferring to the embryo through placenta. In the current study, the outbreak of HPV16 infection among pregnant women was

evaluated as 29.78% (14/47), while it was 6.38% (3/47) for HPV18. Most of the conducted studies have also reported HPV16 as the most prevalent type among pregnant women with a variable frequency ranged from 5.5% to 65%.

In various studies carried out in several countries, HPV has been introduced as one of the most prevalent infection factors during the pregnancy period. As an example, the prevalence of this virus in pregnant women has been reported 6.5% in Spain, 12.5% in Japan, 19.6% in Netherland, 35.14% in Poland and 85.5% in Brazil (Castellsagué *et al.*, 2009; Moreira *et al.*, 2009; Takakuwa *et al.*, 2006). The differences in prevalence can be due to the different frequencies of HPV infection among general population of each country. Meanwhile, the method used to detect the infection must also be taken into consideration. Therefore, high prevalence and significance of this virus must not be ignored among the people who take high risk sexual activities.

PCR technique has been used as the method to detect HPV DNA in many studies. The sensitivity of PCR has been emphasized by many researchers and it has been suggested as the method of choice as compared to the pathological methods with a high level of false negative results such as pap-smear.

It seems that the availability of an anti-HPV vaccine to protect the prevalent and high risk types of this virus can decrease the risk of premature delivery and abortion in pregnant women. However, the high outbreak of HPV16 and HPV18 throughout the world show that the available vaccines have not been successful in preventing the infection. Therefore, new immunization plans are needed to be implemented in high-prevalent areas.

Conclusion

The information obtained from this research showed that papilloma virus, with a relatively high prevalence, is present in the virginal samples of the pregnant women of the studied society. Meanwhile, only 1 case with premature delivery and 3 cases with abortion were observed, which all were positive for HPV16. Transfer of HPV from mother to embryo during pregnancy is considered as a serious risk factor to threat the embryo's life. Therefore, screening of individuals before pregnancy can prevent the infection and complications resulted from it.

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