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ORIGINAL ARTICLE

The occurrence of genital types of human papillomavirus in normal pregnancy and in pregnant women with pregestational insulin dependent diabetes mellitus

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Abstract

OBJECTIVE: The aim of the study was to evaluate the prevalence of human papillomavirus, both types of a low oncogenic risk (HPV 6, 11) and a type carrying a high oncogenic risk (HPV 16) in the genital tract of the pregnant patients, their venous blood, the cord blood and the oral cavities of the neonates. Normal pregnant women and pregnant women with insulin dependent diabetes mellitus (IDDM) diagnosed before pregnancy were included in the study.

DESIGN: The study group consisted of 15 pregnant women aged 22 to 32 years with IDDM diagnosed before pregnancy. The control group consisted of 30 patients aged 18 to 38 years, with normal pregnancy. The DNA of HPV types 6, 11 and 16 was studied in the discharge from the cervical canal, the maternal venous blood, the cord blood and the buccal smear obtained from the neonates. To detect of viral DNA the PCR was used.

SETTING: A university teaching hospital delivering approximately 2000 women annually.

RESULTS: Human papillomavirus (HPV) was found in 12 (26.7%) of the 45 pregnant women. Of the 15 patients with pregestational IDDM the DNA of HPV was detected in four (26.7%) of the patients. The DNA of HPV types 6 and 11 was found in three (20%) patients. The DNA of HPV type 16 was detected in one pregnant patient in the study group (6.67%). Of the 30 control patients, HPV DNA was detected in eight (26%). In two (6.6%), infection with oncogenic and non-oncogenic types of HPV was diagnosed. The DNA of HPV types 6 and 11 was found in six (20%) subjects. Of the 30 control patients, the DNA of HPV type 16 was detected in four (13.3%). The transmission of HPV from HPV-positive mother to fetus was found in 50% of cases.

CONCLUSIONS: (i) There was a similar level of occurrence of HPV infections in pregnant women with IDDM when compare with normal pregnancy. (ii) High pecentage of HPV transmission from mother to neonate was determined. (iii) The cesarean section probably does not protect the neonate from HPV infection. (iv) There is a suggestion that fetus may be affected by HPV infection during intrauterine life.

Introduction

Cervical cancer is the second [1] or, according to some authors the first, most common malignancy in women worldwide and it accounts for 60% of all gyneacologic malignancies. Nowadays, cervical cancer is increasingly more commonly considered to be essentially a sexually transmitted disease. The association between cervical cancer and its aetiologic factor – human papillomavirus (HPV) – has been extensively studied for over 20 years. The correlation between HPV infection and cervical cancer seems to be even stronger than that between smoking and lung cancer [21]. The presence of HPV DNA has been confirmed in 90% of cases of diagnosed cervical cancer, while the remaining 10% might involve so far unrecognized types of HPV [5]. The time from HPV infection to the development of invasive cancer is usually 15 years [12]. The incidence of HPV infection has dramatically increased in recent years, the peak being between ages of 15 and 35 years [6]. So far over 100 types of HPV have been identified, causing lesions in the reproductive organs and skin [10]. HPV finding in a particular part of the genital tract suggests that the virus has invaded the entire reproductive system of the patient [6]. Genital types of HPV are transmitted mainly sexually, but HPV may be also spread by oral contact, by fingers or during the neonate's passage through the infected birth canal [10]. However, it is a matter of debate whether in cases of HPV infection of the infant by the mother, the virus is transmitted during the fetal passage through the infected birth canal or the infection develops in utero.

In pregnancy, the immune system is physiologically suppressed [1]. Recently, there have been numerous reports describing the immunosuppressive role of hormones: oestrogens, progesterone, chorionic gonadotropin and chorionic somatomammotropin. Alpha-fetoprotein and prostaglandins type E also posses immunosuppressant properties.

Additional disorders of specific and nonspecific immunity occur in pregnant diabetics. Ischaemia and anoxia are clinically the most important pathogenetic events which predispose to infection in patients with diabetes mellitus (DM).

The processes by which specific immune antibodies and mediators of inflammation are formed are also impaired. Disorders of specific immunity involve both humoral and cellular types of response. Neutrophils are the first-line defence cells in the early phase of the inflammatory response. In patients with insulin-dependent diabetes mellitus (IDDM) granulocytes exhibit impaired chemotaxis and reduced phagocytic and bactericidal properties while the count of T lymphocytes, which predominate in the normal cervical epithelium, is decreased.

The aim of the study was to evaluate the prevalence of human papillomavirus, both types of a low oncogenic risk (HPV 6, 11) and a type carrying a high oncogenic risk (HPV 16) in the genital tract of the pregnant patients, their venous blood, the cord blood and the oral cavities of the neonates. Normal pregnant women and pregnant women with IDDM diagnosed before pregnancy were included in the study.

Material and methods

Forty-five pregnant patients were included in the study. The study group consisted of 15 pregnant women aged 22 to 32 years with IDDM diagnosed before pregnancy. DM was classified according to White. Class B diabetes was diagnosed in five patients (33.3%), Class C – in eight (53.3%), Class D in one (6.7%) and Class RF in one patient (6.7%). The control group consisted of 30 patients aged 18 to 38 years, with normal pregnancy. The women from both study and control groups received antenatal care and gave birth at the 1st Department of Obsterics and Gynaecology, Medical University of Warsaw, in the years 2000–2002.

The characteristics of the two groups are summarized in Table 1.

The DNA of HPV types 6, 11 and 16 was studied in the discharge from the cervical canal, the maternal venous blood, the cord blood and the buccal smear obtained from the neonates. Pap smears were obtained from the cervix in the 3rd trimester of pregnancy during routine check-up examinations in the Antenatal Clinic. Two sterile swabs were used to obtain the smears. The obtained material was placed in 10 ml of physiological saline. At delivery, 10 ml of maternal venous blood and 10 ml of cord blood were collected into heparinized test tubes. A buccal smear was obtained using two sterile swabs from each neonate in the 2nd day of life. The obtained material was placed in 10 ml of physiological saline.

To detect viral DNA the PCR (polimerase chain reaction) was used.

Table 1. Characteristics of patients.

	Study group N (%)	Control group N (%)
age		
< 25 years old	3 (20)	9 (30)
25-30 years old	9 (60)	13 (43)
31-35 years old	3 (20)	6 (20)
>35 years old	0	2 (6.7)
Ed Education level		
• elementary	8 (53.3)	5 (16.7)
• secondary	6 (40)	12 (40)
• university	1 (6.7)	13 (43.3)
Smoking	1 (6.7)	4 (13)
Oral contraception before pregnancy	2 (13.3)	9 (30)
	x ± SD	x ± SD
Height [cm]	161.1 ± 4.11	164.9 ± 5.96
Weight before pregnancy [kg]	60.7 ± 11.97	56.7 ± 8.66
Weight gain during pregnancy [kg]	10.3 ± 2.97	15.8 ± 8.66
Gestational age [weeks]	36.5 ± 1.2	39.4 ± 1.27

x – medium value
SD – standard deviation

Each patient enrolled in the study gave her informed consent.

The statistic analise was performed on the base of Fisher test.

Results

Human papillomavirus (HPV) was found in 12 (26.7%) of the 45 pregnant women.

Of the 15 pregnant patients with IDDM six (40%) were primiparous and nine (60%) were multiparous. In six (40%) of the patients, delivery by caesarean section was performed before 37 weeks gestation for obstetric indications. Nine of the patients (60%) gave birth between 37 and 39 weeks gestation: two vaginal deliveries (22.2%) and seven caesarean sections (77.8%) because of obstetric, general medical or ophthalmologic indications.

Of the 15 patients with pregestational IDDM the DNA of HPV was detected in four (26.7%) of the patients.

The DNA of HPV types 6 and 11 was found in three (20%) patients (Table 2). The women were aged 24 to 29 years, two were primiparous and one was a multipara. The three deliveries were by caesarean section. The patients' characteristics are summarized in Table 3.

The DNA of HPV type 16 was detected in one pregnant patient in the study group (6.67%) – Table II. She was a 28-year-old multipara referred for caesarean section at 37 weeks gestation because of hypertension, previous caesarean section and pregestational DM.

Of the 30 controls, 24 (80%) were primiparous and six (20%) were multiparous. All gave birth between 38 and 42 weeks gestation: 22 vaginal deliveries (73.3%) and eight deliveries (26.7%) by caesarean section for obstetric indications.

Of the 30 control patients, HPV DNA was detected in eight (26%) – Table IV. In two (6.6%), infection with oncogenic and non-oncogenic types of HPV was diagnosed.

The DNA of HPV types 6 and 11 was found in six (20%) subjects (Table IV). These were women aged 21–33 years with deliveries between 38 and 41 weeks of gestation – vaginal delivery in four cases and delivery by caesarean section because of obsteric indications in two cases.

Of the 30 control patients, the DNA of HPV type 16 was detected in four (13.3%), see Table IV. The patients aged 24–30 years, gave birth between 38 and 40 weeks gestation (vaginal delivery in two cases and delivery by caesarean section due to obsteric indications also in two cases).

Discussion

The DNA of human papillomavirus (HVP) was identified in 12 (26.7%) of the 45 pregnant women included in the study, i.e. four IDDM patients (26.7%) and eight controls (26.7%).

Although women with IDDM are known to be more susceptible to new infections and even chronic infections may be more promptly reactivated than in healthy subjects [9], HPV infection was not found to be more prevalent in our group of pregnant patients with pregestational IDDM. The rate of HPV infection was the same in IDDM patients and healthy controls (26.7%). In the study by Hietanen et al. [9], HPV types 11, 16 and 18 were found in 3.2% of pregnant IDDM patients compared to 5.9% of normal pregnant women. Interestingly, in that study the percentage of HVP-positive patients was lower in the DM group than in normal pregnant women. In our groups of DM patients and healthy controls, the HPV-infection rate was the same (26.7%) and much higher than reported by Hietanen et al. [9]. Those authors believe that the low incidence observed in the two groups can be accounted for by the absence of risk factors. The women had had no sexually transmitted diseases in the past, were all married and had not had multiple sexual partners.

Table 2. The presence of HPV 6/11 and 16 in pregnant women with IDDM

	Patient	Cervical discharge		Venous blood		Cord blood		Buccal smear from the neonates	
		6/11	16	6/11	16	6/11	16	6/11	16
1	BP	---	+	---	+	---	+	---	---
2	AA	---	---	---	---	---	---	---	---
3	BC	+	---	---	---	+	---	---	---
4	EM	---	---	---	---	---	---	---	---
5	DW	---	---	---	---	---	---	---	---
6	JP	---	---	---	---	---	---	---	---
7	MP	---	---	---	---	---	---	---	---
8	AZ	---	---	---	---	---	---	---	---
9	RM	---	---	---	---	---	---	---	---
10	AK	+	---	+	---	---	---	---	---
11	JK	+	---	---	---	---	---	+	---
12	MS	---	---	---	---	---	---	---	---
13	EG	---	---	---	---	---	---	---	---
14	EM	---	---	---	---	---	---	---	---
15	BZ	---	---	---	---	---	---	---	---

The estimated presence of HVP DNA in pregnant women reported by different authors varies widely. The percentage of positive findings depends on the method of assay and the number of particular primers used in the study.

So far, studies on the incidence of HPV infection in pregnancy have yielded controversial results, demonstrating either a higher incidence of HPV infection in pregnant women [7, 16, 20, 22] or no differences compared to non-pregnant women [11, 24]. Kjellberg et al. [12], however, claim that pregnancy may protect against HPV infection.

There is a correlation between the percentage of positive findings and the age of patients. According to published studies [13], HPV infection is the most common at the age 20–25 years and the incidence falls over age 35 years.

Evander et al. [4], who studied a group of young women aged 19–25 years, detected HPV infection in 21% of the subjects while a year later only 8.3% remained HPV-positive. In most cases, especially in young women, HPV infection is short-lasting and transient while the memory of the immune system is likely to protect the woman against further infection with the same types of HPV [4]. This mechanism is thought to be one of the causes of less frequent finding of HPV after the age of 30 years, or according to some authors after the age 35 years. It has been reported that the types associated with

Table 3. The characteristic of patients with IDDM, HPV 6 and 11

Patient	Type of DM (by White)	Gestational age	Mode of delivery	Indication to cesarean section
BC	B	37	CS	obstetric
AK	C	36	CS	obstetric
JK	RF	34	CS	obstetric

CS – caesarean section

Table 4. The presence of HPV 6/11 i 16 in uncomplicated pregnancy .

	patient	Cervical discharge		Venous blood		Cord blood		Buccal smear from the neonates	
		6/11	16	6/11	16	6/11	16	6/11	16
1.	WL	+	---	---	---	---	---	+	---
2.	AW	---	---	---	---	---	---	---	---
3.	IG	---	---	---	---	---	---	---	---
4.	A R-C	---	---	---	---	---	---	---	---
5.	SB	---	---	---	---	---	---	---	---
6.	E P-Z	---	---	---	---	---	---	---	---
7.	EC	---	---	---	---	---	---	---	---
8.	J B-R	---	---	---	---	---	---	---	---
9.	EP	---	---	---	---	---	---	---	---
10.	RJ	---	---	---	---	---	---	---	---
11.	EW	+	---	+	---	---	---	---	---
12.	RF	---	---	---	---	---	---	---	---
13.	KL	---	---	---	---	---	---	---	---
14.	MG	---	---	---	---	---	---	---	---
15.	MM	---	---	---	---	---	---	---	---
16.	AB	---	---	---	---	---	---	---	---
17.	GN	---	---	---	---	---	---	---	---
18.	MP	---	+	---	---	---	---	---	+
19.	BŚ	---	---	---	---	---	---	---	---
20.	AZ	+	+	---	+	---	+	---	+
21.	KC	---	---	---	---	---	---	---	---
22.	DK	---	---	---	---	---	---	---	---
23.	MD	---	---	---	---	---	---	---	---
24.	AZ	---	---	---	---	---	---	---	---
25.	BO	---	---	---	---	---	---	---	---
26.	BO	+	---	---	---	---	---	---	---
27.	MD	---	---	---	---	---	---	---	---
28.	MI	+	---	+	---	+	---	+	---
29.	RJ	---	+	---	---	---	+	---	+
30.	KN	+	+	---	---	---	---	---	---

a higher oncogenic risk are more frequently detected in subjects over 30 years of age [8]. This may be due to chronic infection caused by highly oncogenic types of HPV while infection produced by non-oncogenic types of HPV resolves spontaneously leaving its imprint on the memory of the immune system.

In the present study, of the 12 HPV-positive pregnant patients, 58% – the highest percentage – were aged 25–30 years. A higher incidence of HPV infection in women below 30 years of age has been observed by Eppel et al. [3] and Tenti et al. [26].

The transmission of HPV from HPV-positive mother to fetus was found in 50% of cases (six neonates). Two deliveries were vaginal (33.3%) and four (66.7%) by caesarean section. The findings were compared with those reported by other authors. The rates of vertical transmission have varied widely: 5.29% [28], 27% [2], 30% [26], 31.6% [18], 33.3% [20], 37% [19] and 39.7% [27], but were lower than our finding of 50%.

The presence of HPV in the oral cavities of neonates delivered by caesarean section, in the maternal venous blood and in the cord blood suggests that the transmission of HPV may have occurred before birth.

Summing up, we have not found more frequent HPV infection in pregnant patients with pregestational DM in spite of the immune suppression while the vertical transmission of papillomavirus from HPV-positive mother to fetus seems very likely.

Conclusions

1. There was a similar level of occurrence of HPV infections in pregnant women with IDDM when compare with normal pregnancy.
2. High percentage of HPV transmission from mother to neonate was determined.
3. The cesarean section probably does not protect the neonate from HPV infection.
4. There is a suggestion that fetus may be affected by HPV infection during intrauterine life.

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