Prevalence of human papilloma virus infection in pregnant Turkish women compared with non-pregnant women

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Summary

Purpose of Investigation: We aimed to find a prevalence of human papilloma virus (HPV) in order to define the 100 genotypes and subset of 14 oncogenic genotypes in pregnant Turkish women and to compare these with non-pregnant women. *Methods:* Cervical thin-prep specimens were obtained from 164 women in the first trimester pregnancy and 153 non pregnant women. *Results:* 29.2% of pregnant versus 19.6% of non-pregnant Turkish women had at least one of the 100 types of HPV infection - a statistically significant difference. The rate of 14 high-risk HPV genotype infections was significantly higher in pregnant (14.6) compared to non-pregnant Turkish women (9.6%). *Conclusions:* Pregnant Turkish women are at higher risk for all HPV infections including high-risk cervical cancer genotypes.

Key words: Human papillomavirus; Cervical cancer; Turkish women; Prevalence; Genotyping.

Introduction

Statistical analyses released from the World Health Organization (WHO) suggest that cervical cancer is the second most common cancer in women worldwide [1-3]. It is estimated that each year approximately 493,000 new cases are diagnosed and 274,000 women die from cervical cancer worldwide [4]. The presence of HPV DNA in cervical tissues has implicated HPV as a causative agent in genital condylomatas, in lower female genital tract intraepithelial neoplasias, such as cervical intraepithelial neoplasia (CIN), and in invasive cervical carcinomas [5]. It has been demonstrated that HPV DNA can be detected in approximately 99% of all invasive cervical cancers [6]. In addition, HPV DNA is almost always present in condylomatas and high-grade dysplasias, such as CIN III [7]. HPV types 6 and 11 are known to induce exophytic condylomatas affecting the anogenital mucosa and lower vagina [8]. A subset of HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are regarded as oncogenic, or high-risk, HPV viral types. This subset represents the predominant HPV genotypes detected in high-grade intraepithelial lesions (CIN II and III) and in carcinomas of the lower female genital tract [6-9]. A basic understanding of HPV epidemiology is required to comprehend the role of various HPV types in the development of cervical cancer and to design effective vaccine strategies against the virus. Different populations may harbor varying HPV genotypes in the genital tract [6]. Thus far, the pregnant and non-pregnant Turkish population has not been studied regarding their prevalence of 100 HPV genotypes. Before utilizing HPV vaccines for a particular population, it is imperative to have relevant HPV genotyping data to provide an optimal vaccine to provide the best possible care for that population. For primary prevention, the approach taken was to develop an HPV vaccine and, recently, HPV prophylactic vaccines have become available in many countries including Turkey. These vaccines are type-specific (for HPV 6, 11, 16 and 18) and protection against cancer is expected to be in the 65-75% range, depending on the distribution of HPV genotypes in the population [10]. For secondary prevention, type-specific HPV testing has been proposed as an additional biomarker to stratify women according to risk for precancerous lesions and cancer [11]. This study provides the baseline data that will be accessible to insure that this population can be appropriately included in vaccine trials in the future.

Material and Methods

Over a 1.5-year time period, 317 cervical samples were collected from pregnant and non-pregnant Turkish women attending the Medico-social Unit of Istanbul University and Sisli Etfal Training and Research Hospital outpatient clinic. Ethical Committee approval of the hospitals was obtained prior to sample collection. Patients presenting at the Gynecology and Obstetrics Clinic for a routine physical examination volunteered to participate in the study. The participants included were sexually active with no previous histological diagnosis or treatment and were seeking cervical cancer screening. A history collection and physical examination were performed on patients, and for conventional Pap smears, samples were prepared on a glass slide. The Paps were diagnosed using the Bethesda system (TBS) in which the following terms are used: atypical squamous cells of undetermined significance (ASCUS), low-grade and high-grade squamous intraepithelial lesion (LSIL, HSIL) [12]. ***DNA isolation:*** Genomic DNA was isolated from the thin prep and biopsy samples according to a standard salting-out protocol. The quality of the DNA isolation was tested with the amplification of the beta-globin gene using the following primers: Globin-F: 5'- GAA GAG CCA AGG ACA GGT AC-3' and Globin-R: 5'-

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CAA CTT CAT CCA CGT TCA CC-3'. The amplification of 270 bp product showed the success of isolation. In case of failure the isolation was repeated. ***HPV detection and genotyping:*** In the present study we used a PCR-based assay to detect and genotype human papillomaviruses (HPV) in mucosal samples. For the detection of HPV, nested-PCR was applied to amplify the consensus MY09/11 region of HPV with MY09/11 and GP5+/6+ primers [13]. The amplification products were visualized in EtBr stained agarose gel electrophoresis. The presence of 150 bp products indicated HPV infection. ***Multiplex PCR:*** For HPV genotyping, after amplification of the E6/E7 oncogene region of HPV using consensus E6/E7 primers, nested multiplex PCR with type-specific primers was used to genotype each 100 HPVs including 14 high-risk HPVs (16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68) [14]. The amplification products were separated in EtBr stained agarose gel electrophoresis. ***Sequencing:*** In patients who tested negative for high-risk HPV, the GP5+/6+ PCR products were sequenced to determine the genotype of HPV. The fragments were sequenced with automated sequencer ABI 3130 PRISM (Applied Biosystems). The resulting sequences were aligned with the Blast program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). ***Statistical analysis:*** We performed statistical calculations using SPSS Version 13.0 for Windows. The chi-square test was performed to assess the statistical significance of differences in the prevalence of HPV infection in pregnant and non-pregnant women and to assess differences in frequency of HPV infection among four seasonal groups; p values of less than 0.05 were considered significant (95% confidence interval).

Results

Mean ages of women in the groups were similar. Parity and gravidity did not differ in either group (Table 1). Thirty non-pregnant Turkish women out of 153 (19.6%) had at least one of the 100 HPV genotypes in their cervical region. Forty-eight women out of 164 in the first trimester gestation were HPV positive (having at least one of the 100 HPV genotypes), which means a high prevalence of HPV (29.2%) in pregnant Turkish women. On the other hand, HPV infection in pregnancy was significantly higher than in non-pregnant women ($p < 0.005$). The prevalence of high-risk HPV infection (14 genotypes) was 14.6% and 9.8% in pregnant and non-pregnant women of our population, respectively, and the difference was significant ($p < 0.05$). Of the pregnant women, 12.1% were infected by high-risk HPV genotype 18 or 16 which were included in the vaccination against HPV infection. Of the non-pregnant women 7.7% were infected by genotype 16 or 18 and the difference was significant ($p = 0.01$). Multiple infection rates in both groups were not different and were very low. Only one pregnant women had three HPV types and two women in the non-pregnant group had two different HPV genotypes together. Two patients in the pregnant group had ASCUS in their cervical cytology; one had genotype 18 and the other had genotype 31 HPV infection. There was no abnormal cervical cytology in the non-pregnant group.

Table 1. — *Demographic properties of the groups.*

	Mean age	Parity	Gravidity
Pregnant women	30.56 ± 7.74	2.4 ± 1.2	2.8 ± 1.4
Non-pregnant women	33.25 ± 8:71	2.8 ± 1.3	3.2 ± 1.5

Table 2. — *Distribution of high-risk HPV genotypes in the groups.*

	HPV						
	16	18	31	33	45	56	58
Pregnant women	12	8	1	2	–	1	–
Non-pregnant women	7	5	1	–	1	–	1

Conclusions

The overall prevalence of HPV infection considering non-pregnant Turkish women was 19.6% which is similar to the American-Indian and Asian population; Bell *et al*. found the prevalence of HPV to be 21.25% in American-Indian women and Li *et al*. found a 22% prevalence of HPV infection in the Northern Chinese population with normal cytology [15, 16]. However this overall prevalence of HPV in our population is relatively high compared to other worldwide studies [17]. Stockman *et al*. found the overall prevalence of HPV to be 45.3% in a French population study which is very high compared to ours [18]. Not only the overall prevalence but also the prevalence of high-risk HPV genotypes were so different in these studies which clearly shows the importance of reginal and ethnic variation in HPV.

Previous studies have indicated a seasonal correlation of HPV infection [19, 20]. However, in our study there was no correlation between HPV infection and seasonal variation.

Two prophylactic virus-like particle-based vaccines (one bivalent vaccine against HPV16 and HPV18 and a quadrivalent vaccine against HPV16/18/6/11) have demonstrated efficacy (90-100%) against persistent infection with targeted types when administered in a three-dose schedule to women who are uninfected with those types [21, 22]. The quadrivalent vaccine is also efficacious in preventing related high-grade cervical lesions, with the results from Phase III trials of the bivalent vaccine awaited [21]. However, because vaccine-induced protection is probably relatively specific for targeted types [23], vaccination will not replace the need for Pap screening programs. Therefore, the potential effectiveness of the vaccine in reducing the burden of Pap abnormalities and cancer will be dependent on local epidemiology. This is why we investigated the prevalence of 14 high-risk HPV genotypes in pregnant and non-pregnant women including type 16 and 18 against which vaccines would be effective, and we found a high prevalence of these in both groups. Thus we concluded that HPV vaccine in the Turkish population, especially before pregnancy, would be highly preventive for cervical cancer.

Why are HPV genotypes, including 14 high-risk genotypes, significantly higher in pregnant women? The reason may be due to an attenuated immune system in pregnancy. A woman may be exposed to genital HPV infection many times but most HPV infections could be

eradicated by her immune system in her normal lifetime. However in pregnancy there is hormonal depression of immune reactions. Another reason may be psycho-social factors in that many couples may decrease the frequency of sexual intercourse due to fear of losing their baby, especially in the first three months of gestation, which may increase multipartner behavior in males.

Bell *et al*. found that the incidence of HPV infection was inversely correlated with age. In younger women (< 24 years) HPV infection was significantly higher (41%, $p < 0.005$) compared to all other age groups [15]. We did not investigate the correlation of age with HPV prevalence because our group of pregnant women was already restricted by reproductive age. However a relatively high HPV prevalence may be partially due to the relatively young ages in the pregnant group and also in the control group.

Multiple infection rates by different HPV genotypes were extremely lower than other populations studied worldwide [17].

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