RESEARCH ARTICLE

Pre-vaccination Prevalence and Genotype Distribution of Human Papillomavirus Infection among Women from Urban Tunis: a Cross-sectional Study

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Abstract

Background: To estimate the pre-vaccination distribution of human papillomavirus (HPV) types among women from urban Tunis. Materials and Methods: A total of 611 women aged 18-69 years were enrolled in three local gynaecological outpatient departments. All underwent a gynaecological examination with Pap test and dry swab for HPV detection and typing performed by linear array genotyping test (Roche). Cytological examination was conducted on conventional Pap smears. Results: HPV DNA was found in 6.5% of the women; the most frequent HPV types were HPV 16 and HPV 11 at 3.27% and 1.96%, respectively. The second most frequent high risk (HR) HPV type was HPV 58 (0.82%) followed by HPV 18, HPV 31 and HPV 33 found in only 0.33% of women. Single infections with HPV types, targeted by the quadrivalent vaccine (6, 11, 16, and 18), were detected in 3.6 % of the study patients (55% of positive women). HPV infection was found in 3.83% of women with normal cytology and in 47.4% of women with cytological abnormalities. No statistically significant trend in prevalence by age group emerged for any HPV type or for high or low risk types. Conclusions: These data show a relatively low prevalence of HPV infection in women from urban Tunis with a high proportion of HPV16 and HPV58. This should be considered in the upcoming screening programs and vaccination strategy.

Keywords: Human papillomavirus - pap smears - urban Tunis - vaccination strategy

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Introduction

The introduction of vaccination against Human papillomavirus (HPV) infection in many countries should reduce considerably the prevalence of cervical cancer and cervical intra-epithelial neoplasia. In developing countries, where screening programs are not effectively implemented, HPV vaccination will be particularly attractive and cost effective, but may be difficult to introduce and to evaluate because epidemiologic data on the distribution of HPV infection and the cervical cancer burden prior to HPV vaccine are scanty.

The available studies on HPV infection in Tunisia and other North African countries are scarce and mostly show preliminary data and a limited number of participants (Hammouda et al., 2005; Abdel et al., 2006). In Tunisia, most published data were focused on prostitutes and showed a high prevalence of HPV infection with high risk types and a high prevalence of HPV 58 (Hassen et al., 1999; 2003; De Marco et al., 2006; Znazen et al., 2010).

A national screening program and a vaccination strategy to prevent cervical cancer are currently under development in Tunisia but data to support a targeted strategy is lacking (Sancho-Garnier et al., 2013). To determine an effective strategy for HPV vaccination and cancer prevention, the laboratory of Pathology at the Pasteur Institute of Tunis, member of the Who HPV LabNet for Eastern Mediterranean region (Unger et al., 2009), conduct a survey to evaluate the distribution, the conducted instead of conduct and the cytology status of HPV infection in healthy females from urban Tunis.

Materials and Methods

667 women aged 18 to 69 years old were enrolled during their regular visits to three local outpatient departments of gynecology in two hospitals and one family planning clinic, in urban Tunis. At the enrolment, informed consent was obtained from all participants. The women were screened for cervical cancer by conventional Pap smears and HPV test. Pregnant women, menstruating women, those with hysterectomy, and those who refused

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the HPV test were excluded. The ethic committee of the Pasteur Institute of Tunis approved the study.

A questionnaire administered by clinicians at the three facilities was used to collect data from the women on socio-economic status and sexual partners.

During gynaecological examination, cervical scrapes for conventional Pap smears were obtained using Ayre's spatula and then fixed with hair spray. A dry cotton swab from the cervical transformation zone was collected, placed in a sterile tube and refrigerated at 4°C (Shah KV et al., 2001). All samples were placed on wet ice and then shipped within the same day of collection to the Department of Pathology.

Cytological analysis

Pap smears were examined by two experienced pathologists and classified in accordance with Bethesda system (2001) in normal, inflammatory, infectious, atypical squamous cells of undetermined significance (ASC-US); low squamous intra-epithelial lesion (LSIL); or high squamous intra-epithelial lesion (HSIL).

HPV detection and typing

All the steps of detection and typing were carried out in the WHO HPV LabNet regional reference Laboratory Who Eastern Mediterranean region. The cotton swabs were suspended in 300 μl PBS and then DNA isolation was carried out with QIAamp DNA Mini and Blood Kit (QIAGEN, Germany) following the manufacturer's instructions.

Samples were tested for HPV DNA by LA amplification and genotyping test (Linear Array HPV Genotyping Test, Roche Diagnostics, Mannheim, Germany). The Linear Array genotyping test (Roche) detects 37 anogenital HPV genotypes including 17 high-risk HPV types (HR) 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59 66, and 68b (ME180) (HPV68a cannot be detected by the PGMY primers), 73 (MM9), and 20 low risk HPV types (LR) 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 82 (MM4), 82 (IS39 subtype), 83 (MM7), 84 (MM8), and 89 (CP6108). Amplification and genotyping were in accordance with Roche Diagnostics instructions. Because women can harbor one or more HPV type infection, multiple infections with at least one HR-HPV type were considered as high risk infection.

All the steps of HPV testing were blinded to the cytological results.

Statistical analysis

Sample size calculation was performed using Epitable command (EpiInfo6 software): using the following estimations: Precision=2.5%, point prevalence=10% and α=5%. The sample size estimation was about 553 subjects.

HPV prevalence standardized by age was estimated on the basis of the female population of Tunisia and of the world standard population. Statistical significance was reported using a χ^2 test for categorical variables and the t test for continuous variables. Odds ratios (OR) and 95% confidence intervals (CI) were computed using unconditional binary logistic regression (SPSS 20.0). Association between HPV test and cytology results was assessed by χ^2 test ($p \leq 0.05$).

Results

Patient characteristics

After cytological examination and DNA testing, 56 women were excluded (inadequate cytology, 31 cases and inadequate DNA, 25 cases). The ages of the remaining 611 women were not significantly different from the excluded group.

Included women were aged 18-69 years (mean age 39 years, standard deviation: 9.52). Most of them were married (95.4%) with middle socio-economic status (73.9%).

Cytological findings

No cytological abnormalities were shown by Pap smears in 573 women. Smears results were distributed as follows: 338 normal, 57 atrophic, 138 inflammatory and 42 infectious (other than HPV).

Cytological lesions were found in 38 Pap smears (6.2%): 10 atypical squamous cells of undetermined significance (1.6%), 24 low squamous intra-epithelial lesion (3.9%) and 4 high squamous intra-epithelial lesion (0.7%). Women with abnormal cytological findings underwent a colposcopal examination, confirmatory biopsies, and treatment, when necessary.

Type-specific distribution of HPV DNA

Among the 611, 40 women were positive for HPV DNA (6.5%; 95% CI: 4.5-8.4). Positivity was distributed in 26 single and 14 multiple infections. In total, 64 HPV infections were found with 15 different viral types. High-risk (HR) HPV types were found in 4.9% of the study patients (75% of positive women).

The most frequent HR types were HPV 16 (3.27%; 95% CI: 1.8-4.7) followed by HPV 58 (0.82%; 95% CI: 0.1-1.53), HPV 18, HPV 31 and HPV 33 (0.33%; 95% CI: 0.13-0.78) (Table 1). The most frequent LR types were HPV 11 (1.96%; 95% CI: 0.8-2.9) and HPV 6 (0.65%; 95% CI: 0.02-1.29). Overall, single infections with HPV

Table 1. Prevalence of HPV Types with Corresponding 95% CI among Women from Urban Tunis

HPV type	Proportion among HPV infections%	Prevalence [CI 95%]
6	6.3 (4/64)	0.65 [0.02-1.29]
11	18.8 (12/64)	1.96 [0.86-3.06]
16	31.3 (20/64)	3.27 [1.86-4.68]
18	3.1 (2/64)	0.33 [0.13-0.78]
31	3.1 (2/64)	0.33 [0.13-0.78]
33	3.1 (2/64)	0.33 [0.13-0.78]
42	3.1 (2/64)	0.33 [0.13-0.78]
51	1.6 (1/64)	0.16 [0.16-0.48]
53	1.6 (1/64)	0.16 [0.16-0.48]
54	1.6 (1/64)	0.16 [0.16-0.48]
56	1.6 (1/64)	0.16 [0.16-0.48]
58	7.8 (5/64)	0.82 [0.1-1.53]
61	6.3 (4/64)	0.65 [0.02-1.29]
73	6.3 (4/64)	0.65 [0.02-1.29]
89 (CP6108)	4.7 (3/64)	0.49 [0.06-1.05]
Total infections	100.0	

Table 2. Prevalence of HPV Infection in Women with Normal and Abnormal Cytology in Relation to HPV Risk Type and Multiplicity of Infection

		Cytology									
		Without abnormalities					Abnormal				
		normal	atrophic	inflammatory	infectious	Total	ASCUS	LSIL	HSIL	Total	Total
N	338	57	136	42	573		10	24	4	38	611
HPV-	328	54	133	36	551 (96.2%)		9	11	0	20 (52.6%)	571(93.4%)
HPV+	10 (2.9%)	3(5.3%)	3(2.2%)	6(14.3%)	22(3.8%)		1(10%)	13(54.2%)	4(100%)	18(47.4%)	40(6.5%)
	HR[1] single	3		1	2	6 (1.1%)		8	3	11 (28.9%)	17 (2.8%)
	mixed[3]	4	1	1	1	7 (1.2%)	1	4	1	6 (15.8%)	13 (2.1%)
	LR [2] single	3	2	1	3	9 (1.6%)					9 (1.5%)
	mixed							1		1 (2.6%)	1 (0.2%)
	Single infection										
	6			1	1	2 (0.4%)					2 (0.3%)
	11	2	1		2	5 (0.9%)					5 (0.8%)
	16	2		1	1	4 (0.7%)		7	3	10 (26.3%)	14 (2.3%)
	18				1	1 (0.2%)					1 (0.2%)
	33							1		1 (2.6%)	1 (0.2%)
	61	1				1 (0.2%)					1 (0.2%)
	73	1				1 (0.2%)					1 (0.2%)
	CP6108		1			1 (0.2%)					1 (0.2%)
	Mixed infection										
	11;16;42;56;CP6108	1				1 (0.2%)					1 (0.2%)
	11;16;58		1			1 (0.2%)					1 (0.2%)
	11;54;61							1		1 (2.6%)	1 (0.2%)
	11;58						1	2		3 (7.9%)	3 (0.5%)
	11;58;61							1		1 (2.6%)	1 (0.2%)
	16;33			1		1 (0.2%)					1 (0.2%)
	16;CP6108	1				1 (0.2%)					1 (0.2%)
	18;51								1	1 (2.6%)	1 (0.2%)
	31;42;73	1				1 (0.2%)					1 (0.2%)
	6;16;31;73							1		1(2.6%)	1 (0.2%)
	6;16;73				1	1 (0.2%)					1 (0.2%)
	61;53	1				1 (0.2%)					1 (0.2%)

*1: HR-HPV: high risk HPV types, 16. 18. 31. 33. 51. 53. 56. 58. 73; 2: LR-HPV: low risk HPV types, 6. 11. 42. 54. 61. CP6108; 3: Mixed infections were considered as high risk if they harbour at least one HR-HPV type.

types included in quadrivalent vaccine (6, 11, 16, and 18) were detected in 3.6 % of the study patients (55% of HPV positives).

Combined cytological and HPV typing results

The 38 women with cytological abnormal Pap smears were highly associated with HPV infection ($p<0.05$). This association was relevant for any viral type. HPV DNA was found in 47.4% of these women including 10% in atypical squamous cells of undetermined significance, 54.2% in low squamous intra-epithelial lesion and 100% in high squamous intra-epithelial lesion. HR-HPV types were also significantly associated with cervical abnormalities ($p<0.001$), they were involved in 92.3% of positive low squamous intra-epithelial lesion and in all cases of high squamous intra-epithelial lesion. The most common HR-HPV types in this group were HPV 16 and HPV 58, they were found respectively in 61.1% and 22.2% of positive women with cervical abnormalities.

The 573 women without cervical abnormalities had an HPV prevalence of 3.8% corresponding to 55% of HPV positives. HR-HPV types were found in 59% of them. The most frequent HR-HPV types were HPV16 (1.57%) and HPV 73 (0.52%). Women with infectious cytology showed a HPV prevalence of 14.3% (OR= 5.4 95%CI: 1.97-14.54). Half of these women had HR-HPV types (Table 2).

Age and HPV infection

HPV infections were found in all age groups.

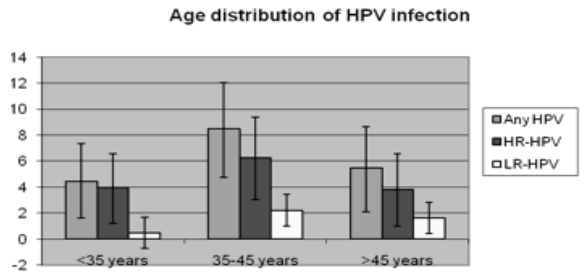

Figure 1. Age-specific Prevalence of Cervical HPV Infection And Corresponding 95% CI in the Study Group

Prevalence standardized by age on the basis of the female population of Tunisia and of the world standard population was 5.95% and 5.47% respectively. Women aged 35 to 45 year had the highest prevalence of HPV and HR-HPV infections. Low risk (LR) HPV types were most often found in women over 55 years old. Nevertheless, no statistically significant trend in prevalence by age group emerged for any HPV type nor for high or low risk types (Figure 1).

Discussion

This study is the largest survey on the distribution of HPV infection in Tunis. It shows important differences from the global pattern and those of neighbouring countries. The prevalence of HPV infection was low without a decline with increasing age and HPV 58 was

the second most frequent HR-HPV type

Age-standardized HPV prevalence in this study was lower than reported data in other North African countries (Hammouda et al., 2005; Abdel et al., 2006; Hammouda et al., 2011) and in several areas from Asia, Europe and Sub-Saharan African countries (Clifford et al., 2005; De Vuyst et al., 2009; Akcali et al., 2013). It was also lower than previous reports from the region of Sousse in the center of Tunisia (14%) (Hassen et al., 1999; 2003). This low prevalence could denote a difference in the distribution of HPV infection between regions or be related to monogamy as 95% of the studied women had one partner.

The age distribution of HPV infection shows the highest prevalence in women aged 35-45 years but without statistically significant difference between age groups. This age pattern was reported previously in several developing countries like Nigeria (Clifford et al., 2005), Algeria (Hammouda et al., 2011), China and Thailand (Dai et al., 2006; Laowahutanont et al., 2014). In his Meta analysis, Smith (2008) showed that such pattern was common in countries with low HPV prevalence and attributed it in a large part to the HPV persistence or the high rate of reactivation of HPV infection. Other authors suggested the role of new infection acquisition after the age of 30 years (Castle et al., 2005).

The distribution of HR-HPV types was slightly different from the global pattern. HPV 16 was like the majority of reports the most frequent type but with high proportion (50%) exceeding those reported in low HPV risk population (Clifford et al., 2005; Smith et al., 2008; Olivera-Silva et al., 2011). This proportion was high in women under 35 years and decreases with age suggesting that women aged 35 and over acquire immunity against HPV 16. The second most frequent HR HPV was type 58 found in 12.5% of HPV positives. This pattern was consistent with previous results from Tunisia (De Marco et al., 2006) and several areas from Asia and Africa (Xi et al., 2003; Takehara et al., 2011), it emphasizes the geographic diversity in the distribution of HPV infection and the necessity to improve screening for HPV 58 in Tunisian women, in order to better assess this genotype prevalence and its association with cervical abnormalities.

Single infections with HPV types targeted by the quadrivalent vaccine (HPV 6, 11, 16 and 18) were found in 55% of HPV positives (3.6% of the study group). This means that the introduction of a vaccination program could prevent almost half of the HPV infections in Tunisian women.

Women with normal cytology showed a HPV prevalence of 3.8%. Although this prevalence was low compared with reported series from Africa (7.3%-37.1%), America (4.6%-42.2%) and Europe (8.5%-22.7%) (Bruni et al., 2010), it represents 55% of HPV positives. This high rate of infra-cytologic cases could be related to several factors. Firstly, the use of Ayre's spatula for cervical sampling has been suggested to be less efficient than other devices because of the low yield of representative cells and koilocytes (Martin-Hirsch et al., 1999). Secondly, Pap smears may show hemorrhage or high inflammation level with numerous neutrophils, probably resulting in misdiagnosis of HPV infection lesions. Indeed, in this study, women with infectious cytology showed a high level of HPV infection (14%). These results suggest that using more efficient cervical devices and better assessing the infectious cytology in future screening programs could enhance the sensitivity of the screening.

The prevalence of HPV infection among low squamous intra-epithelial lesion was 54.2%. Reported prevalence in the same group ranged from 33.3% in Africa to 97.5% in America (Evans et al., 2006; Hammouda et al., 2011). The most frequent high risk HPV types were HPV 16 and HPV 58 found respectively in 21.1% and 5.3% of low squamous intra-epithelial lesion. HPV16 was 1.5-fold more common and HPV58 4.8-fold more common in abnormal cytology relative to normal one. These results are consistent with other reports (Xi et al., 2003; Bruni et al., 2010) and confirm the role of HPV 16 and 58 in the development of cervical abnormalities.

In conclusion, based on these results, the introduction of HPV vaccine could reduce considerably the prevalence of HPV infection. In addition, a national screening program including high quality cytology and a HPV testing will improve the sensitivity of the screening and reduce significantly the incidence of cervical cancer.

These are results from urban Tunis which will be extended to other regions to better assess the prevalence and the distribution of HPV types in Tunisia.

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