Prevalence of human papillomavirus infection in women in Turin, Italy

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Abstract

Human papillomavirus (HPV) is recognised as necessary for the development of cervical cancer. An age-stratified random sample of 1013 women, aged 25–70 years, participating in the organised cervical screening programme in Turin, Italy was tested for 36 HPV types using polymerase chain reaction (PCR) with the general primers GP5+/GP6+. The overall HPV prevalence was 8.8%. High-risk types were found in 7.1% of women and multiple infections in 1.1%. HPV-16 was the most common type (32.6% of HPV-positive women). HPV prevalence (any type) was 13–14% at ages 25–39 years, 11.5% at age 40–44 years, and approximately 5% among older women. After age-adjustment, HPV prevalence was significantly increased in single *vs* married, (Odds Ratio (OR) = 2.23; 95% Confidence Interval (CI): 1.28–3.89) and decreased in parous *vs* nulliparous women (OR = 0.49; 95% CI: 0.31–0.78). However, the association with marital status and parity was restricted to women less than 45 years of age. In conclusion, overall, the female population of Turin showed an HPV prevalence that is intermediate compared with worldwide levels.
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Keywords: Human papillomavirus; Cervical cancer; Cervical dysplasia; Prevalence; Age

1. Introduction

Infection with high-risk types of human papillomavirus (HPV) is found in virtually all cases of cervical cancer [1] and is considered a necessary factor in invasive cervical cancer [1,2]. This finding has stimulated interest in HPV DNA testing as a means of screening for cervical cancer [3] and in the development of prophylactic and therapeutic vaccines against HPV [4].

Before vaccines or HPV-based cervical screening can be introduced at a population level, information is needed on the age- and type-specific prevalence of HPV infection in representative samples of the female population. Few such studies have been conducted [5–13], particularly in Southern Europe [14,15], and no large population-based survey of the prevalence of type-specific HPV is available for Italy.

In Turin, an organised screening programme for cervical cancer has been active for many years. All female residents aged 25–64 years are invited to have a Papanicolau (Pap) smear every 3 years, independent of their history of spontaneous screening [16]. Comparisons of the socio-demographic features of the women who do

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and do not participate in this programme have not shown any substantial differences [17] and, therefore, screening participants can be considered to be a reasonably representative sample of the Turin population.

We estimated the prevalence of cervical HPV infection in this population by means of a cross-sectional study, one of a number of HPV prevalence surveys being conducted by International Agency for Research on Cancer (IARC) on different continents.

2. Methods

2.1. Study population

Cervical cell samples were obtained from all women who came after invitation (between April and June 2002) for cytological analysis within the framework of the Turin organised cervical screening programme during a pilot project on the use of liquid-based cytology. Of these women, 6556 (89.4%) agreed to participate in the present study and gave their written informed consent.

A random sample of stored specimens was selected, excluding those from 559 women who (a) were pregnant, (b) had undergone a hysterectomy or (c) whose smear was unsatisfactory (e.g., lack of endocervical cells). Conversely, the presence of cytological abnormalities was not considered a basis for exclusion. We sampled approximately 100 women in each 5-year age group between 25–29 years and 65–70 years. Each age group included a proportion of women who had not been screened previously, and this reflected the proportions of women in the Turin population in the same age groups who had previously been estimated never to have been screened [16]. All women aged over 64 years who participated were included in our sample. Overall, samples from 1025 women were analysed.

2.2. Human papillomavirus DNA detection and typing

Cervical cells were obtained with a Cervex brush and placed in Cytorich® medium. After one thin-layer slide had been prepared, the remaining material was centrifuged at 600 rotations per minute (rpm) for 5 min. The preserving liquid was then discarded, and the pellet was re-diluted in 150 μl of Cytorich® medium and transferred to Eppendorf tubes, which were stored at −20 °C.

HPV DNA was detected by polymerase chain reaction (PCR) with the consensus primer set GP5+/GP6+, which targets a highly conserved fragment of the L1 open-reading frame. Specific HPV groups were detected with high-risk and low-risk cocktail probes in a PCR–immunoassay procedure. Final HPV typing was performed with a non-radioactive reverse line blot system with specific oligoprobes for the most frequent HPV types.

Aliquots of 100 μl of each specimen were boiled for 10 min at 100 °C and immediately cooled on ice. The first aliquot of 10 μl of a given extract was screened with a *β-globin* PCR, to check the DNA quality and competence; a second 10 μl aliquot was used for HPV DNA detection and typing. The *β-globin* gene amplification was positive in 1013/1025 samples (98.8%). The 12 *β-globin*-negative samples were excluded from the HPV analysis.

In the *β-globin*-positive samples, HPV DNA was detected using the GP5+/GP6+ PCR assay, which allows identification of a broad spectrum of genital HPV types [18]. PCR positivity was assessed by hybridisation of PCR products in an enzyme immunoassay (EIA) using two HPV oligoprobe cocktails that together contain the following 36 HPV types: 6, 11, 16, 18, 26, 31, 33–35, 39, 40, 42–45, 51–59, 61, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, and CP6108 [6,19]. The sensitivity and specificity of EIA detection were determined previously using dilution lines of cloned HPVs or cervical smears in which these types were identified in earlier studies [6,20].

Final genotyping for the individual HPV types was achieved with a recently described reverse line blot hybridisation assay [19] with specific probes. The system is based on a miniblotter (MN45, Immunetics, Cambridge, MA, USA) that spots up to 37 different oligoprobes which are covalently bound to negatively charged membranes in parallel lines. Forty-two biotinylated products from the GP5+/GP6+ PCR were spotted in lines perpendicular to those containing the oligoprobes. Hybridisation was performed for 1 h at 42 °C in the miniblotter and hybrids were visualised by a peroxidase-labelled streptavidin conjugate that interacts with biotinylated DNA. Final detection was done by enhanced chemiluminescence (ECL, Amersham, Bucks, England). HPV types were classified as high-risk and low-risk according to Muñoz and colleagues [21]. Special precautions were taken to minimise false-positive results in the PCR, as described in detail elsewhere [22].

2.3. Explanatory variables

The information collected from each woman during the gynaecological visit included parity, concomitant pregnancy, previous gynaecological treatments and current use of oral contraceptives. The socio-demographic characteristics of the women and, for married women, of their husbands, were determined by means of record linkage with data in the Turin Population Registry, which is virtually complete and highly reliable. Additional information, such as the occupations of women and their husbands, was obtained through linkage with the records of the 1991 population census using a previously validated method [23]. The latter record linkage was possible for 84% of our study women.

2.4. Statistical analysis

Odds ratios (ORs) for positivity for HPV were computed by means of unconditional logistic regression models. The approximate 95% Confidence Intervals (CIs) of the ORs were computed from Wald-type standard errors. In selected cases, a test of the overall effect of categorical variables was computed by a likelihood ratio χ^2 comparing models with and without the considered variables. All *P* values are two-sided. The ORs were adjusted for age (5-year groups) and for additional variables, as specified.

3. Results

3.1. Human papillomavirus prevalence and distribution of types

HPV infection was found in 89 of the 1013 women eligible for the DNA analysis (8.8%; 95% CI: 7.0–10.5). The prevalence in the age range 25–70 years was 8.8% when standardised by age on the basis of the Turin population in 1998 and 9.5% when standardised on the basis of the world standard population. HPV DNA was found in 78 of the 997 women with normal cytological specimens (7.8%) and in 11/16 (68.8%) of those with abnormal cytology. These included 2 cases with histologically-confirmed cervical intraepithelial neoplasia (CIN) grade III and 3 cases with histologically confirmed CIN I. All were positive for high-risk HPV types (Table 1).

We identified a total of 103 infections from 24 different HPV types, and 11 women (12.4% of those infected) carried multiple types. High-risk types (Table 1) were found in 72 women (7.1% of study women and 80.9% of infected women). HPV 16 was by far the most common type (29 women infected, alone or in conjunction with other types), followed by HPV 66 (10 women), HPV 45 (7 women) and HPV 31 (6 women). Type 18 was found in only one woman. Some 17 women were infected with low-risk types only (1.7% of study women, 19.1% of infected women). HPV 42 (8 cases) and HPV 81 (4 cases) were the most common low-risk types.

The prevalence of HPV (any type) was 13–14% among women aged 25–39 years, 11.5% in those aged 40–44 years (Fig. 1 and Table 2) and only approximately 5% in the older age groups. Despite some fluctuations from one group to another, the downwards trend in HPV prevalence was compatible with a linear decrease: the linear effect by single year was statistically significant ($\chi^2_{1df} = 15.29$; $P < 0.0001$) and adding age in 5-year groups increased the fit only marginally (likelihood ratio $\chi^2_{8df} = 6.25$; $0.75 < P < 0.50$). Although low-risk types were rarer than high-risk types at any age, the age pattern was somewhat different and prevalence of low-risk types reached a small peak at 40–44 years (3.8%).

3.2. Determinants of the prevalence of human papillomavirus infection

After adjustment for age, the prevalence of HPV was slightly increased in women with the lowest (no recognised diploma) or highest (university degree) educational levels in comparison with a primary school degree. However, none of these differences attained statistical significance (Table 2). Place of birth (South or North of Italy) was unrelated to HPV prevalence (data not shown). Prevalence was significantly increased among the few women who reported not having a toilet at home (OR = 3.51; 95% CI: 1.07–11.51).

The prevalence of HPV slightly increased among women whose husbands had no educational degree (Table 2), but was not related to the husband's age nor to the age difference between spouses (data not shown).

An OR of 2.23 (95% CI: 1.28–3.89) was found for single (never married) compared with married women. HPV was detected significantly less frequently in parous than in nulliparous women (OR = 0.49; 95% CI: 0.31–0.78), but among parous women, the number of children was not significantly associated with HPV positivity (Table 2). Age at marriage was unrelated to HPV prevalence (Table 2). HPV infection was, however, inversely associated with the age at first birth (OR for age $\geqslant 30$ *vs* <20 = 0.28; 95% CI: 0.10–0.84), and this trend persisted after adjustment for education, occupation and parity. Current use of oral contraceptives was associated with an OR of 1.36 (95% CI: 0.73–2.52).

3.3. Age trend by marital status and parity

In order to further elucidate the combined effect of age, marital status and nulliparity, which were the strongest correlates of HPV infection in this study, we evaluated HPV prevalence by age separately in single women (never married), nulliparous married women and parous married women (Table 3). At age 25–44 years, single women had a higher prevalence of HPV than parous married women (OR = 3.24; 95% CI: 1.61–6.51), while the prevalence of HPV among nulliparous married women was intermediate. The prevalence of HPV was similar among all strata at $\geqslant 45$ years of age.

4. Discussion

The prevalence of HPV DNA in our study in Turin was 8.8% in women aged 25–70 years, and decreased to 7.8% after exclusion of women with abnormal cervical cytological findings. The peak prevalence was 13.9%, in the age group 35–39 years (i.e., later than in most populations studied so far). A similar HPV prevalence was found in women aged 24–64 years in a case-control study conducted within the cervical screening

Table 1
Human papillomavirus (HPV) types detected in 1013 women in Turin, Italy, 2002

HPV	Cytological finding					
	Normal[a]		Abnormal[b]		All	
	No. of women	(% of all women)	No. of women	(% of all women)	No. of women	(% of all women)
Negative	919		5[e]		924	
Positive	78	(7.8)	11	(68.8)	89	(8.8)
High-risk types[c]	61	(6.1)	11	(68.8)	72	(7.1)
Low-risk types[d]	17	(1.7)	0		17	(1.7)
6	1	(0.1)	0		1	(0.1)
11	2	(0.2)	0		2	(0.2)
16	24	(2.4)	2[f]	(12.5)	26	(2.6)
18	1	(0.1)	0		1	(0.1)
31	2	(0.2)	3[e]	(18.8)	5	(0.5)
33	1	(0.1)	1[g]	(6.3)	2	(0.2)
35	1	(0.1)	0		1	(0.1)
39	3	(0.3)	0		3	(0.3)
42	4	(0.4)	0		4	(0.4)
43	1	(0.1)	0		1	(0.1)
45	5	(0.5)	1[g]	(6.3)	6	(0.6)
51	1	(0.1)	1[e]	(6.3)	2	(0.2)
52	2	(0.2)	0		2	(0.2)
54	2	(0.2)	0		2	(0.2)
56	2	(0.2)	0		2	(0.2)
58	3	(0.3)	0		3	(0.3)
61	1	(0.1)	0		1	(0.1)
66	6	(0.6)	0		6	(0.6)
68	1	(0.1)	1[e]	(6.3)	2	(0.2)
70	1	(0.1)	0		1	(0.1)
72	2	(0.2)	0		2	(0.2)
81	3	(0.3)	0		3	(0.3)
Total single	69	(6.9)	9	(56.3)	78	(7.7)
16,42	2	(0.2)	0		2	(0.2)
16,66	1	(0.1)	0		1	(0.1)
31,53	1	(0.1)	0		1	(0.1)
33,42, **53,58**	0		1[g]	(6.3)	1	(0.1)
42,**66**	0		1[e]	(6.3)	1	(0.1)
45,56	1	(0.1)	0		1	(0.1)
51,59,66	1	(0.1)	0		1	(0.1)
52,68	1	(0.1)	0		1	(0.1)
56,81	1	(0.1)	0		1	(0.1)
58,66	1	(0.1)	0		1	(0.1)
Total multiple	9	(0.9)	2	(12.5)	11	(1.1)
Total	997		16		1013	

[a] Includes benign cellular changes.

[b] Atypical squamous cells of undetermined significance (7 cases) or more severe cytology.

[c] Alone or with low-risk types.

[d] In the absence of high-risk types.

[e] No histologically-confirmed cervical intraepithelial neoplasia (CIN).

[f] Histologically confirmed CIN 3.

[g] Histologically confirmed CIN 1.

programme in Florence, in central Italy. Infection with any of six high-risk HPV types (HPV 16, 18, 31, 33, 52 and 58) was found in 5.1% of 332 archival smears from women without CIN II, or more severe lesions [24]. The overall prevalence of HPV DNA among 3305 cytologically-negative women aged 15–70 years who participated in cervical screening in The Netherlands was 4.6%, and the rate peaked at 20% at age 25–29 years [6]. The incidence of invasive cervical cancer in that country in 1993–1997 (world-standardised rate 6.6/ 100 000) [25] was also similar to that in Turin (6.8/ 100 000).The rate of detection of HPV DNA in a random population sample of 1000 women aged 20–29 years with normal cytological appearance in Copenhagen, Denmark, was 15% [26], which is similar to the prevalence of 13% that we found in Turin among women aged 25–29 years. The prevalence of HPV among 878 women aged 20–49 years with normal cervical smears who were sampled randomly from general practice lists in Ontario, Canada, was 13%, with a peak of
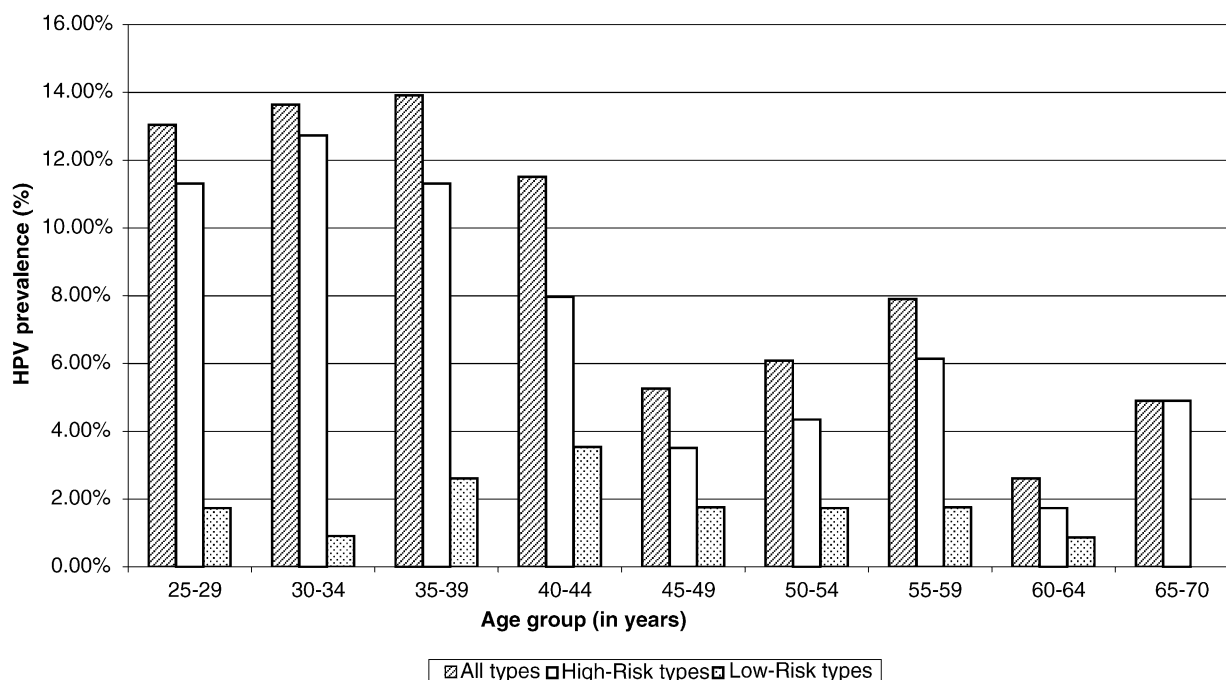
Fig. 1. Prevalence of human papillomavirus infection by age group in Turin, Italy, 2002.

24% among those aged 20–24 years [7]. Much lower HPV prevalence was observed in other southern European countries, like Spain (3%) [15] and Greece (2.5%) [14].

Community-based surveys on continents other than Europe and North America have shown a broad range of prevalence, from 2% in Hanoi, Vietnam [10], to 26% in Ibadan, Nigeria [13], among women aged 15–74 years.

Most of the cases of cervical infection with HPV in our study were single infections with high-risk types. The frequency of multiple infections was lower than in other populations [6], especially Latin American ones in whom HPV infection is substantially more frequent [5,11]. In our study, half of the infections were due to types 16, 66, 45 and 31, in decreasing order. The frequency of HPV 16 was high (32.6% of HPV-positive women), while type 18 was almost absent. Low-risk types were rare, but, as observed in other areas [5,6,12], they represented a larger proportion of infections in middle-aged women than in young women.

We found a limited effect of education and occupation on the prevalence of HPV, although both low and very high social classes appeared to be related to a slightly increased risk. A direct association between HPV prevalence and years of education was observed in a few studies, although the finding was not always statistically significant [9,10,12,27].

Current use of oral contraceptives was not associated with an increased risk for HPV infection in our study, but we had no information on the duration of such use. An association between HPV DNA and oral contraceptive use, after adjustment for sexual habits, was reported in some studies [7,10,26,28,29], but not in others [8,27,30,31], and whether oral contraceptives affect the incidence or prevalence of HPV infection remains unclear [32].

Together with age, marital status and parity were the strongest determinants of HPV prevalence in our study. As in other studies [10,12,26,27,33], we found that nulliparous woman had a higher frequency of HPV DNA than parous women; nevertheless, there was no clear trend according to the number of pregnancies.

Part of the variation in HPV prevalence by age in this population is plausibly related to changes in sexual behaviour of study women and their sexual partners, depending on ageing itself and on birth cohort. The similarity in HPV prevalence in nulliparous married women and single women below age 45 years suggests that sexual habits among women or their husbands in Turin change after the birth of the first child as well as after marriage. However, this does not rule out a role for age-related factors other than HPV exposure, such as acquired immunity. We also observed some reduction in HPV prevalence with increasing age among married parous women. Unfortunately, it was not possible to assess the effect of sexual behaviour directly, as we did not collect information on sexual habits: this is the most relevant limitation of the present study. In a study among women from a minority population in inner New York City, who were tested by Southern blot hybridisation (overall HPV prevalence = 19.8%), the inverse

Table 2
Prevalence ORs of human papillomavirus (HPV) infection and corresponding 95% CI for age and selected characteristics. Turin, Italy, 2002

Characteristic	HPV−	HPV+	(%)	Age-adjusted OR (95% CI)[a]
Age (years)				
25–29	100	15	(13.04)	1
30–34	95	15	(13.64)	1.05 (0.49–2.27)
35–39	99	16	(13.91)	1.08 (0.51–2.30)
40–44	100	13	(11.50)	0.87 (0.39–1.92)
45–49	108	6	(5.26)	0.37 (0.14–0.99)
50–54	108	7	(6.09)	0.47 (0.17–1.10)
55–59	105	9	(7.89)	0.57 (0.24–1.37)
60–64	112	3	(2.61)	0.18 (0.05–0.64)
65–70	97	5	(4.90)	0.34 (0.12–0.98)
Education				
University degree	61	12	(16.44)	1.91 (0.81–4.51)
Secondary or high school	561	56	(9.08)	0.95 (0.50–1.83)
Primary school (5 years)	258	16	(5.84)	1
None	44	5	(10.20)	1.44 (0.49–4.27)
Marital status				
Married	684	53	(7.19)	1
Single (never married)	145	29	(16.67)	2.23 (1.28–3.89)
Widowed	62	4	(6.06)	1.43 (0.46–4.46)
Divorced	33	3	(8.33)	1.41 (0.41–4.88)
Husband's education[b]				
University degree	45	2	(4.26)	1.00 (0.20–4.96)
Secondary or high school	406	35	(7.94)	1.91 (0.84–4.34)
Elementary school	192	8	(4.00)	1
None	30	4	(11.76)	3.02 (0.83–10.93)
Age at marriage (years)				
<20	79	8	(9.20)	1
20–24	340	28	(7.61)	0.81 (0.39–1.69)
25–29	214	11	(4.89)	0.47 (0.19– 1.14)
⩾ 30	113	10	(8.13)	0.82 (0.33–2.03)
Linear trend				$\chi^2_{1df} = 0.07; P = 0.79$
Age at first birth (years)				
< 20	78	13	(14.29)	1
20–24	268	22	(7.59)	0.50 (0.24–1.06)
25–29	221	9	(3.91)	0.25 (0.10–0.61)
⩾ 30	107	5	(4.46)	0.28 (0.10–0.84)
Linear trend				$\chi^2_{1df} = 7.12; P = 0.0076$
Number of children				
Nulliparous	250	40	(13.79)	1
Parous (total)	674	49	(6.78)	0.49 (0.31–0.78)
1 child	256	23	(8.24)	0.58 (0.33–1.02)
2 children	303	18	(5.61)	0.40 (0.22–0.74)
⩾ 3 children	115	8	(6.50)	0.48 (0.21–1.09)
Current oral contraceptive use				
Yes	94	16	(14.55)	1.36 (0.73–2.52)
No	830	73	(8.08)	1

Only married women included for husband's education or occupation; only parous women included for information on children; only married or widowed women included for age at marriage.
OR, Odds Ratio; 95% CI, 95% Confidence Interval.
  [a] Adjusted for age (5-year groups).
  [b] Information missing for 15 women.

relationship between age and HPV prevalence persisted after adjustment for sexual behaviour, and sexual habits had a limited effect within age strata [34]. The role of acquired immunity in different populations can be expected to vary according to the frequency of HPV infection.

Table 3
Prevalence of human papillomavirus by marital status, parity and age group. Turin, Italy, 2002

	25–44 years of age				45–70 years of age				All ages			
	HPV−	HPV+	(%)	OR[a] (95% CI)	HPV−	HPV+	(%)	OR[a] (95% CI)	HPV−	HPV+	(%)	OR[a] (95% CI)
Parous married	225	23	(9.27)	1	436	24	(5.22)	1	661	47	(6.64)	1
Nulliparous married	63	9	(12.50)	1.61 (0.69–3.78)	55	4	(6.78)	1.37 (0.45–4.12)	118	13	(9.92)	1.46 (0.74–2.85)
Single women	106	27	(20.30)	3.24 (1.61–6.51)	39	2	(4.88)	0.88 (0.19–3.94)	145	29	(16.67)	2.47 (1.37–4.44)

OR, Odds Ratio; CI, Confidence interval.
a Adjusted by 5-year age within each age category.

Our study population was representative of the female population of Turin, as we sampled women participating in the organised cervical screening programme, to which all women between 25 and 64 years are actively invited every 3 years. Approximately 35% of the women invited for screening during the study period accepted. This corresponds to the compliance to invitation previously observed [16]. However overall coverage in the Turin population is about 75% as a result of both spontaneous and organised screening [16]. In a previous study of the factors associated with attendance for screening in Turin [17], we found no significant association between participation in the screening programme and education, marital status or place of birth. In order to make the present study population more representative of that of Turin, we included in each age group a proportion of women who had never been screened before. We obtained samples from almost 90% of the women attending the screening programme during the study period, and only a small proportion of samples (1.2%) were inadequate for HPV testing. For HPV DNA detection and typing, we applied a highly sensitive PCR-based method that was used in most other IARC surveys [6,9–12,24], thereby improving comparability with the results from other countries. However, Turin is an industrial city in North-western Italy, and therefore our HPV findings cannot be considered representative of those in other parts of Italy where no information is yet available. Indeed, the detection rate of cervical intraepithelial lesions varies widely in different areas of Italy [35]. This suggests that HPV prevalence may vary as well.

In conclusion, our study of the female population of Turin showed a high HPV prevalence compared with other southern European populations. In particular, the relatively high prevalence of HPV infection in all cohorts of women born after 1960 emphasises the importance of strengthening screening efforts to avoid a future increase in cervical cancer.

Conflict of interest statement

No conflict of interest.

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