AGE-SPECIFIC DISTRIBUTION OF HPV GENOTYPES IN WOMEN FROM THE SOUTHWESTERN REGION OF THE REPUBLIC OF NORTH MACEDONIA

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Abstract: Patient age is a well recognised determinant of both the natural history of human papillomavirus infection and the probability of cytological progression, however, the majority of investigations address this relationship only at the level of the broad distinction between single- and multiple-type infection, and the age distribution of individual genotypes remains insufficiently characterised. The present study was undertaken to address this question among HPV-positive women identified through primary cervical screening. The investigation was conducted at the Centre for Public Health Bitola between February 2019 and August 2023. Three hundred women positive for human papillomavirus were tested for twenty-one genotypes by real-time polymerase chain reaction and stratified by cytology into a normal finding, low-risk lesions and high-risk lesions. For each genotype, the mean age of carriers and non-carriers was compared, and the distribution of carriers across five age groups was assessed. Mean age increased significantly with lesion severity, from 31.1 years in the normal group to 35.1 in low-risk and 37.1 in high-risk lesions ($p = 0.000112$). At the genotype level, statistically significant age signatures were identified. Several genotypes occurred more frequently among the youngest women: type 73 carriers had a mean age of 28.3 years, with 59.1% aged 24 or younger ($p < 0.0001$); type 82 was likewise more frequent in this age group (54.5% aged ≤ 24; $p = 0.0018$); and types 59 and 53 also demonstrated significant age structures. In contrast, type 31 carriers were significantly older, and type 68 carriers were over-represented in the 55-to-64 age group ($p = 0.012$). Type 16 showed a significant age-group distribution ($p = 0.04$) but no significant difference in mean age, whereas type 18 exhibited no age preference. These reproducible genotype-specific age signatures indicate that certain genotypes behave as transient infections of younger women while others persist into older age. It is recommended that genotype-resolved, age-aware interpretation be incorporated into risk-stratification algorithms and into the determination of screening intervals, so that individual genotypes are weighted according to their age profile and oncogenic potential rather than treated as equivalent. The present analysis is based on results derived from a doctoral dissertation and contributes region-specific data from southwestern North Macedonia, where such genotype-resolved age data have not previously been reported.

Keywords: human papillomavirus, genotype, age distribution, cervical screening, risk stratification

1. INTRODUCTION

The association between patient age and human papillomavirus infection is among the most consistently documented patterns in cervical epidemiology. Prevalence reaches its maximum in the years following sexual debut, the majority of infections acquired at this stage are spontaneously cleared, and prevalence subsequently declines with age, frequently with a secondary peri-menopausal increase (Donnikov et al., 2019). Age is likewise associated, in broad terms, with the type of infection, multiple-type infections predominating in younger women and single-type infections in older women (Salazar et al., 2015). The severity of cytological abnormalities also tends to increase with age, reflecting the fact that high-grade lesions arise from long-standing persistent infection, which is more frequently encountered in older women.

Considerably less is established regarding whether this age dependence is uniform across genotypes or whether individual types possess distinct age profiles. The majority of cohort studies aggregate genotypes into high- and low-risk categories, or into single- versus multiple-type infection, and are consequently unable to determine whether carriers of one genotype differ in age from carriers of another. Such differences would be of practical relevance: a genotype occurring predominantly among the youngest women is more likely to represent a transient infection, whereas a genotype persisting into older age may carry greater oncogenic significance per case. Genotype-resolved age data of this nature remain scarce, particularly within the Balkan region.

Accordingly, the present study was designed with three objectives. The first was to examine the relationship between patient age and the severity of the cytological finding within the study population. The second, and principal, objective was to characterise the age profile of each detected genotype, thereby identifying genotypes associated with younger and with older age. The third was to consider the implications of these genotype-specific age profiles for age-aware, genotype-resolved cervical screening.

2. METHODOLOGY

Study design and population

This was a retrospective-prospective, single-point cross-sectional study at the Department of Microbiology, Section of Serology with Molecular Microbiology, of the Centre for Public Health Bitola. The analysis included 300 women positive for human papillomavirus from Bitola, Resen and Kichevo, who attended primary cervical screening with their gynaecologist between February 2019 and August 2023 and had complete genotyping and cytology results. Eligible women were aged 18 to 65 years, not pregnant, without prior hysterectomy and without cervical treatment in the previous six months, and all gave written informed consent. Women with incomplete results, insufficient cellular material, a history of gynaecological cancer, or serious chronic disease were excluded. The study followed the Declaration of Helsinki and had institutional ethics approval.

Genotyping and cytology

From each woman, a cervical swab was taken for viral testing and a conventional Papanicolaou (Pap) smear for cytology at the same visit. Twenty-one genotypes were detected by real-time polymerase chain reaction (DNA-Technology, Russia): the low-risk types 6, 11 and 44 and the high-risk types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82. For the genotype-specific analyses, women in whom a given genotype was detected were **classified as** carriers of that genotype, and the remaining HPV-positive women, in whom it was not detected, were considered non-carriers. The Pap smears were read by a pathologist according to the Bethesda system and grouped into a normal finding (negative for intraepithelial lesion or malignancy), low-risk lesions (atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesions) and high-risk lesions (high-grade squamous intraepithelial lesions, atypical squamous cells where a high-grade lesion could not be excluded, and squamous cell carcinoma).

Statistical analysis

For each genotype, the mean age of carriers and non-carriers was compared by Student's t-test, and the distribution of carriers across the five age groups was tested by the chi-square test, with Fisher's exact test where samples were small. Mean age across cytological groups was compared by analysis of variance with post-hoc Tukey comparisons. A probability below 0.05 was taken as significant.

3. RESULTS AND DISCUSSION

Study population

The study population included 300 women positive for human papillomavirus, with a mean age of 34.4 years (range 18 to 61). The largest proportion of women belonged to the 25 to 34 age group (37.0%), followed by the 35 to 44 group (29.0%), with prevalence declining in the older age groups. By cytology, 96 women (32%) had a normal finding with a positive viral test, 120 (40%) had low-risk lesions and 84 (28%) had high-risk lesions.

Age and cytological severity

Mean age increased progressively with the severity of the cytological finding: 31.1 (± 10.1) years in the normal group, 35.1 (± 9.1) in low-risk lesions and 37.1 (± 9.5) in high-risk lesions. The overall difference was highly significant (analysis of variance, $F = 9.38$, $p = 0.000112$), and post-hoc testing demonstrated that it was attributable to the significantly younger age of women with normal cytology relative to both lesion groups ($p =$

0.0069 and p = 0.000095); whereas women with low-risk and high-risk lesions did not differ significantly from one another (p = 0.29) (Table 1). The same age-related pattern was apparent when women were stratified by a negative or positive smear result: a normal result predominated in the youngest age group (34.4% versus 10.8%; p < 0.0001), whereas positive results were significantly more frequent in the 35 to 44 (p = 0.0031) and 45 to 54 (p = 0.0168) age groups.

Table 1. Mean age by cytological group

Cytological group	n	Mean age ± SD (years)	p value
Normal finding	96	31.1 ± 10.1	F = 9.38
Low-risk lesions	120	35.1 ± 9.1	p = 0.000112
High-risk lesions	84	37.1 ± 9.5	

ANOVA with post-hoc Tukey test. Normal vs low-risk p = 0.0069; normal vs high-risk p = 0.000095; low- vs high-risk p = 0.29.

Source: Authors' own research/data

The increase in mean age with lesion severity agrees with the established understanding that persistent infection, and consequently high-grade lesions, become more common in older women (Tekkesin et al., 2022; Salazar et al., 2015). It confirms that the present cohort conforms to expected epidemiological behaviour, which provides the basis for the subsequent genotype-level analysis that constitutes the principal contribution of the study.

Age signatures according to genotype

When the age structure of the genotypes was considered individually, differences were pronounced (Table 2 and Table 3). The strongest association with younger age was observed for genotype 73, whose carriers had a mean age of 28.3 years compared with 34.9 years among non-carriers (p = 0.0022). 59.1% of type 73 carriers were aged 24 years or younger, whereas only 15.5 percent of non-carriers belonged to that age group (p < 0.0001), and the share in the 25 to 34 range decreased correspondingly to 13.6 percent, compared to 38.9 percent among non-carriers (p = 0.018). Type 59 showed a similar pattern, its carriers being significantly younger than non-carriers (29.7 versus 34.9 years; p = 0.0083). Type 82 showed no significant difference in mean age, but it was significantly more frequent in the youngest age group (54.5% of carriers aged 24 or younger, versus 17.3% of non-carriers; p = 0.0018). Two genotypes showed the reverse pattern. Type 31 carriers were significantly older than non-carriers (mean ages 37.0 and 33.8 years, respectively; p = 0.031), significantly spread across age groups (p = 0.048) and less represented in the 25 to 34 age range (24.5% versus 39.7%, respectively; p = 0.038). Genotype 68 occurred most frequently among the oldest women, being significantly more common in the 55-to-64 age group (11.1% versus 2.9%; p = 0.03) and less common in the 35-to-44 age group (7.4% versus 31.1%; p = 0.0095). Genotype 16, the most prevalent type, showed a significant distribution across age groups (p = 0.04): it was less frequent among the youngest women and more frequent in those aged 25 to 44 years. Its mean age, however, did not differ significantly between carriers and non-carriers. Type 18 was the only genotype to show no age-related pattern at all.

Table 2. Mean age of carriers versus non-carriers for individual HPV genotypes

Genotype	Carriers, mean age ± SD (years)	Non-carriers, mean age ± SD (years)	p value
HPV-6	31.9 ± 10.4	34.6 ± 9.7	0.238
HPV-16	35.6 ± 8.5	34.0 ± 10.2	0.236
HPV-18	32.4 ± 8.9	34.5 ± 9.8	0.350
HPV-31	37.0 ± 10.6	33.8 ± 9.5	0.031
HPV-35	39.6 ± 11.6	34.2 ± 9.7	0.090
HPV-45	36.9 ± 11.7	34.2 ± 9.6	0.250
HPV-51	32.7 ± 10.7	34.6 ± 9.7	0.270
HPV-52	32.3 ± 10.2	34.6 ± 9.7	0.220
HPV-53	33.3 ± 11.7	34.6 ± 9.5	0.440
HPV-59	29.7 ± 8.5	34.9 ± 9.8	0.0083
HPV-66	35.3 ± 10.8	34.2 ± 9.6	0.500
HPV-68	34.3 ± 12.5	34.4 ± 9.5	0.976

HPV-73	28.3 ± 8.8	34.9 ± 9.7	0.0022
HPV-82	31.0 ± 11.4	34.5 ± 9.7	0.240

Student's t-test. Statistically significant differences ($p < 0.05$) were observed for types 31 (older carriers), 59 and 73 (younger carriers).

Source: Authors' own research/data

Table 3. Age-group distribution of carriers for genotypes with a significant age structure (n and % of carriers within each age group)

Genotype	≤ 24	25–34	35–44	45–54	55–64
HPV-73	13 (59.1)	3 (13.6)	6 (27.3)	0	0
HPV-82	6 (54.5)	1 (9.1)	3 (27.3)	0	1 (9.1)
HPV-53	9 (21.4)	20 (47.6)	7 (16.7)	0	6 (14.3)
HPV-16	8 (11.3)	25 (35.2)	27 (38.0)	11 (15.5)	0
HPV-31	10 (18.9)	13 (24.5)	19 (35.8)	6 (11.3)	5 (9.4)
HPV-68	8 (29.6)	10 (37.0)	2 (7.4)	4 (14.8)	3 (11.1)
Whole cohort	56 (18.7)	111 (37.0)	87 (29.0)	35 (11.7)	11 (3.7)

Chi-square / Fisher's exact test. Values are the number of carriers in each age group and the corresponding percentage of all carriers of that genotype. Significant distributions: HPV-73, -82, -53 ($p < 0.01$), HPV-31 ($p = 0.048$), HPV-68 ($p = 0.012$), HPV-16 ($p = 0.04$).

Source: Authors' own research/data

These age-related patterns are biologically coherent. The genotypes occurring most frequently among the youngest women — types 73, 82, 59 and 53 — are also those least represented in older women and, according to the other analyses of this cohort, are not among the predominant types in high-grade lesions. This profile is consistent with transient infections that are acquired shortly after sexual debut and cleared before progression can occur. The persistence of types 31 and 68 into older age points to the opposite situation: infections that are more likely to have survived immune clearance, and that therefore remain detectable in older women not because they are common but because they are durable. The behaviour of the two most oncogenic genotypes is itself informative. Type 18 showed no age preference at all, and type 16, although its distribution across age groups reached significance, was not confined to any single age group and showed no difference in mean age. Since the presence of these two types is not predicted by a woman's age, both warrant equal attention at every age, and their detection should prompt the same vigilance in a young woman as in an older one.

The overall age-related pattern observed in this cohort is concordant with previous reports. Tekkesin et al. (2022) documented the highest infection prevalence in women under 31 years with a subsequent steady decline, and Donnikov et al. (2019), in a large series from the Russian Federation, described the bimodal age curve, with peaks in the youngest and the peri-menopausal women, that underlies the present findings. The contribution of the present study is to break down this overall age curve into its individual genotypes and to show that the dependence on age differs substantially from one genotype to another.

Limitations

The study is subject to several limitations. Its cross-sectional design permits description of the ages at which genotypes are detected but does not allow infections to be followed over time, true persistence cannot therefore be inferred directly from a single time point. Certain genotypes were carried by relatively few women, which limits the precision of the per-genotype age comparisons and indicates that the weaker associations should be interpreted with caution. Finally, the cohort originates from a single region, and confirmation in larger, longitudinal series would strengthen the genotype-specific age signatures described here.

4. CONCLUSION

In this screening cohort, cytological severity increased with age, in agreement with the existing literature. The main contribution of this study is the observation that individual genotypes carry distinct age signatures: types 73, 82, 59 and 53 represent infections of younger women and are most probably transient, whereas types 31 and 68 persist into older age, while the highly oncogenic types 16 and 18 are not confined to any age stratum. Genotype-resolved interpretation, considered jointly with patient age rather than treating all high-risk types as equivalent, may refine risk stratification and the determination of screening intervals, identifying older women carrying persistence-prone genotypes while limiting unnecessary investigation of younger women carrying genotypes that typically undergo spontaneous clearance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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